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- (71) Applicant (for all designated States except US): ANTI-SOMA RESEARCH LIMITED [GB/GB]; West Africa House, Hanger Lane, Ealing, London W5 3QR (GB).
- (72) Inventor; and
- (75) Inventor/Applicant (for US only): YOUNG, Robert, James [GB/GB]; Antisoma Research Limited, West Africa House, Hanger Lane, Ealing, London W5 3QR (GB).
- (74) Agent: THOMAS, Philip, J., D.; Eric Potter Clarkson, Park View House, 58 The Ropewalk, Nottingham NG1 '5DD (GB).

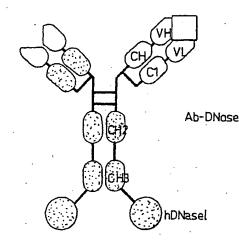
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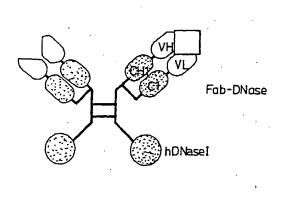
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(54) Title: COMPOUNDS FOR TARGETING





(57) Abstract: A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises a humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity. Preferably, the target cell-specific portion comprises a humanised HMFG-1 antibody or an antigen binding fragment thereof. Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease, e.g. a human DNA endonuclease I. The invention further provides nucleic acids encoding the compounds of the invention, and the use of such compounds in medicine, e.g. in the treatment of cancer.

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## COMPOUNDS FOR TARGETING

The present invention relates to cytotoxic compounds that have a high avidity for, and can be targeted to, selected cells. Specifically, the invention provides compounds comprising a cytotoxic portion having DNA endonucleolytic activity and a target-cell specific portion having specificity for human polymorphic epithelial mucin (PEM).

### 10 Background

The cell-specific targeting of compounds that are directly, or indirectly, cytotoxic has been proposed as a way to combat diseases such as cancer. Bagshawe and his co-workers have disclosed (Bagshawe (1987) Br. J. Cancer 56, 531; Bagshawe et al (1988) Br. J. Cancer 58, 700; WO 15 88/07378) conjugated compounds comprising an antibody or part thereof and an enzyme, the antibody being specific to tumour cell antigens and the enzyme acting to convert an innocuous pro-drug into a cytotoxic The cytotoxic compounds were alkylating agents, e.g. a compound. para-N-bis(2from released mustard benzoic acid 20 chloroethyl)aminobenzoyl glutamic acid by the action of Pseudomonas sp. CPG2 enzyme.

An alternative system using different pro-drugs has been disclosed (WO 91/11201) by Epenetos and co-workers. The cytotoxic compounds were cyanogenic monosaccharides or disaccharides, such as the plant compound amygdalin, which release cyanide upon the action of a β-glucosidase and hydroxynitrile lyase.

In a further alternative system, the use of antibody-enzyme conjugates containing the enzyme alkaline phosphatase in conjunction with the prodrug etoposide 4'-phosphate or 7-(2'-aminoethyl phosphate)mitomycin or a combination thereof have been disclosed (EP 0 302 473; Senter et al (1988) Proc. Natl. Acad. Sci. USA 85, 4842).

Rybak and co-workers have disclosed (Rybak et al (1991) J. Biol. Chem. 266, 21202; WO 91/16069) the cytotoxic potential of a monomeric pancreatic ribonuclease when injected directly into Xenopus oocytes and the cytotoxic potential of monomeric RNase coupled to human transferrin or antibodies directed against the transferrin receptor. The monomeric RNase hybrid proteins were cytotoxic to human erythroleukaemia cells in vitro.

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Other approaches are the *in vivo* application of streptavidin conjugated antibodies followed, after an appropriate period, by radioactive biotin (Hnatowich *et al* (1988) *J. Nucl. Med.* 29, 1428-1434), or injection of a biotinylated mAb followed by radioactive streptavidin (Paganelli *et al* (1990) *Int. J. Cancer* 45, 1184-1189). A pilot radioimmunolocalisation study in non-small cell lung carcinomas was conducted with encouraging results (Kalofonos *et al* (1990) *J. Nucl. Med.* 31, 1791-1796).

Apart from these examples, it is rather more common to see biotinylated antibodies and streptavidin-enzyme conjugates, which are used in enzymelinked immunosorbent assays.

These previous systems have used relatively large antibody-enzyme,

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antibody-streptavidin or antibody-biotin conjugates and may comprise portions of non-mammalian origin which are highly immunoreactive.

We have now devised improved compounds for targeting cells to be destroyed.

# **Summary of Invention**

A first aspect of the invention provides a compound comprising a target cell-specific portion and a cytotoxic portion characterised in that the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof, and the cytotoxic portion has endonucleolytic activity.

By "target cell specific" portion we mean the portion of the compound which comprises one or more binding sites which recognise and bind to polymorphic epithelial mucin (PEM) on the target cell. Upon contact with the target cell, the target cell specific portion is preferably internalised along with the cytotoxic portion. Such internalisation results in the cytotoxic portion being delivered to the cell cytosol, where it has access to the cell's nucleic acid molecules.

The target cell-specific portion of the compounds of the invention comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof.

Polymorphic epithelial mucin, or PEM, is a component of the human milk

fat globule. PEM is expressed by cells in several body tissues and is also found in urine. Significantly, PEM is known to be expressed in epithelial cancer cells, notably in ovarian, gastric, colorectal and pancreatic cancer cells.

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Monoclonal antibodies which will bind to PEM are already known, but in any case, with today's techniques in relation to monoclonal antibody technology, antibodies can be prepared to most antigens. The antigenspecific portion may be a whole antibody, a part of an antibody (for example a Fab or F(ab')<sub>2</sub> fragment), a synthetic antibody fragment (for example a single chain Fv fragment [ScFv]), or a peptide/peptidomimetic or similar. Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "Monoclonal Antibodies: A manual of techniques", H Zola (CRC Press, 1988) and in "Monoclonal Hybridoma Antibodies: Techniques and Applications", J G R Hurrell (CRC Press, 1982) and Antibody Engineering, A Practical Approach, McCafferty, J. et al, ed. (IRL Pres, 1996).

By 'humanised monoclonal antibody' we include monoclonal antibodies having at least one chain wherein the framework regions are predominantly derived from a first, acceptor monoclonal antibody of human origin and at least one complementarity-determining region (CDR) is derived from a second, donor monoclonal antibody having specificity for PEM. The donor monoclonal antibody may be of human or non-human origin, for example it may be a murine monoclonal antibody.

Preferably, both chains of the humanised monoclonal antibody comprise

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CDRs grafted from a donor monoclonal antibody having specificity for PEM.

Advantageously, the CDR-grafted (i.e. humanised) chain comprises two or all three CDRs derived from a donor antibody having specificity for PEM.

Conveniently, the humanised monoclonal antibody comprises only human framework residues and CDRs from a donor antibody having specificity for PEM.

However, it will be appreciated by those skilled in the art that in order to maintain and optimise the specificity of the humanised antibody it may be necessary to alter one or more residues in the framework regions such that they correspond to equivalent residues in the donor antibody.

Conveniently, the framework regions of the humanised antibody are derived from an human IgG monoclonal antibody.

- Methods of making humanised monoclonal antibodies are well-known in the art, for example see Jones et al. (1986) Nature 321:522-525, Riechmann et al. (1988) Nature 332:323-327, Verhoeyen et al. (1988) Science 239:1534-1536 and EP 239 400 (to Winter).
- In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises an humanised HMFG-1 monoclonal antibody or an antigen binding fragment thereof.

HMFG antibodies are raised against human milk fat globule (HMFG), in a delipidated state (see Taylor-Papadimiriou et al., 1981, Int. J. Cancer 28:17-21 and Gendler et al., 1988, J. Biol. Chem. 236:1282-12823). HMFG-1 monoclonal antibodies bind to a particular component of HMFG, namely polymorphic epithelial mucin (PEM). Binding is thought to involve the amino acid sequence APDTR within the twenty amino acid tandem repeats of the muc-I gene product.

Exemplary humanised HMFG-1 antibodies are disclosed in WO 92/04380.

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Advantageously, the target cell-specific portion is an humanised HMFG-1 monoclonal antibody.

In a preferred embodiment of the first aspect of the invention, the target cell-specific portion comprises a fragment of an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), said fragment retaining the antigen binding properties of the parent antibody.

The variable heavy (V<sub>H</sub>) and variable light (V<sub>L</sub>) domains of the antibody

20 are involved in antigen recognition, a fact first recognised by early
protease digestion experiments. Further confirmation was found by
"humanisation" of rodent antibodies. Variable domains of rodent origin
may be fused to constant domains of human origin such that the resultant
antibody retains the antigenic specificity of the rodent parented antibody

25 (Morrison et al (1984) Proc. Natl. Acad. Sci. USA 81, 6851-6855).

That antigenic specificity is conferred by variable domains and is independent of the constant domains is known from experiments involving WO 01/74905 PCT/GB01/01324

the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better et al (1988) Science 240, 1041); Fv molecules (Skerra et al (1988) Science 240, 1038); disulphide-linked Fv molecules (Young et al., 1995, FEBS Lett. 377:135-139); single-chain Fv (ScFv) molecules where the V<sub>H</sub> and V<sub>L</sub> partner domains are linked via a flexible oligopeptide (Bird et al (1988) Science 242, 423; Huston et al (1988) Proc. Natl. Acad. Sci. USA 85, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward et al (1989) Nature 341, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) Nature 349, 293-299.

By "ScFv molecules" we mean molecules wherein the  $V_H$  and  $V_L$  partner domains are linked via a flexible oligopeptide.

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Chimaeric antibodies are discussed by Neuberger et al (1988, 8th International Biotechnology Symposium Part 2, 792-799).

The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments allows for rapid clearance, and may lead to improved tumour to non-tumour ratios. Fab, Fv, ScFv, disulphide Fv and dAb antibody fragments can all be expressed in and secreted from bacteria, such as E. coli, or eukaryotic expression systems such as Yeast or mammalian systems, thus allowing the facile production of large amounts of the said fragments.

Whole antibodies, and F(ab'), fragments are "bivalent". By "bivalent" we

mean that the said antibodies and F(ab')<sub>2</sub> fragments have two antigen combining sites. In contrast, Fab, Fv, ScFv, disulphide Fv and dAb fragments are monovalent, having only one antigen combining site.

Preferably, the target cell-specific portion of the compounds of the invention comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')<sub>2</sub>, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).

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More preferably, the target cell-specific portion comprises a Fab molecule or a F(ab')<sub>2</sub> molecule.

Yet more preferably, the target cell-specific portion comprises an amino acid sequence encoded by at last part of one or both of the nucleotide sequences of Figure 3(a) and (d).

Most preferably, the target cell-specific portion comprises an amino acid sequence encoded by the nucleotide sequence of Figure 3(a) and an amino acid sequence encoded by the nucleotide sequence of Figure 3(d).

Preferably, the target cell-specific portion recognises the target cell with high avidity.

By "high avidity" we mean that the target cell-specific portion recognises the target cell with a binding constant of at least  $K_d = 10^{-6} \, \text{M}$ , preferably at least  $K_d = 10^{-9} \, \text{M}$ , suitably  $K_d = 10^{-10} \, \text{M}$ , more suitably  $K_d = 10^{-11} \, \text{M}$ , yet more suitably still  $K_d = 10^{-12} \, \text{M}$ , and more preferably  $K_d = 10^{-15} \, \text{M}$  or

even  $K_d = 10^{-18} M$ .

Preferably, the target cell-specific portion comprises an antigen binding fragment of an humanised HMFG-1 monoclonal antibody, e.g. an Fab or F(ab')<sub>2</sub> fragment thereof, wherein a hinge region contains a mutation (i.e. wherein the hinge is a variant or hybrid of a naturally occurring hinge). More preferably, the variant hinge comprises the amino acid sequence CCVECPPCPAPE.

By 'cytotoxic portion' we mean a portion having endonucleolytic activity which is toxic to the cell if it is to reach, and preferably enter said cell.

In a preferred embodiment of the first aspect of the invention, the cytotoxic portion has DNA endonucleolytic activity.

Advantageously, the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.

Examples of known DNA endonucleases include bovine DNase I (see Worrall and Conolly, 1990, J. Biol. Chem. 265:21889-21895). Human pancreatic DNase I has also been cloned (see Shak et al., 1990, Proc. Natl. Acad. Sci. USA 87:9188-9192 and Hubbard et al., 1992, New Eng. J. Med. 326:812-815).

25 Preferably, the endonuclease is a mammalian deoxyribonuclease I.

More preferably, the endonuclease is a human deoxyribonuclease I.

Most preferably, the cytotoxic portion comprises the amino acid sequence shown in Figure 2(a) or 2(b).

Preferably, the cytotoxic portion of the compound of the invention is capable of oligomerisation, e.g. dimerisation. Attachment of the target-cell specific portion to a cytotoxic portion capable of oligomerisation provides a method for increasing the number of binding sites to the target cell. For example, if the target cell-specific portion is joined to a portion capable of forming a dimer then the number of target cell-specific binding sites is two; if the target cell-specific portion is joined to a portion capable of forming a tetramer then the number of target cell-specific binding sites is four. The number of target cell-specific binding sites is greater than one and the compounds may therefore have a greater avidity for the target cell than do compounds which only have one target cell-specific binding sites.

It is preferable for the cytotoxic portion of the compound of the invention capable of oligomerisation to contain no interchain disulphide bonds nor intrachain disulphide bonds; to be well characterised; to be non-toxic; to be stable; to be amenable to preparation in a form suitable for pre-clinical or clinical use or be in pre-clinical or clinical use; and for the subunit monomers to have a high affinity for each other, that is they contain one or more subunit binding sites.

Advantageously, the cytotoxic portion is of mammalian, preferably human, origin. The use of the said mammalian proteins as the cytotoxic portion of the compound of the invention is advantageous since such compounds are less likely to give rise to undesirable immune reactions.

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It will be appreciated by those skilled in the art that the cytotoxic portion may be a variant of a naturally occurring endonuclease.

5 By "a variant" we include cytotoxic portions comprising of a naturally occurring endonuclease wherein there have been amino acid insertions, deletions or substitutions, either conservative or non-conservative, such that the changes do not substantially reduce the endonuclease activity of the variant compared to that of the naturally occurring endonuclease. For example, the variant may have increased activity compared to the naturally occurring endonuclease

Such variants may be made using methods of protein engineering and site-directed mutagenesis commonly known in the art (for example, see Sambrook *et al.*, 1989, *Molecular cloning: A Laboratory Manual*, 2<sup>nd</sup> edition, Cold Spring Harbor Laboratory Press, NY, USA).

In an alternative embodiment, the endonuclease is a restriction endonuclease, such as a microbial type II restriction endonuclease.

20 Exemplary type II restriction endonucleases include *BamHI*, *HindIII*, *MspI*, *Sau3AI*, *HinfI*, *NotI* and *EcoRI*.

In another preferred embodiment of the first aspect of the invention, a nuclear localization signal is incorporated into the compound.

Preferably, the nuclear localization signal (NLS) comprises a nuclear localization signal from the SV40 large T antigen (Kalderon *et al.*, 1984, *Cell* 39:499-509), and specifically the amino acid sequence PKKKRKV.

Inclusion of a nuclear localization signal encourages the compound of the invention to gain access to the chromosomal DNA during the periods of the cell cycle when the nuclear membrane is intact, since the nuclear pores are permeable to large molecules incorporating said nuclear localization signal.

In a further preferred embodiment of the first aspect of the invention, the target cell-specific portion and the cytotoxic portion are fused to create a fusion compound.

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By "fusion compound" we include a compound comprising one or more functionally distinct portions, wherein the distinct portions are contained within a single polypeptide chain produced by recombinant DNA techniques. For example, the compound may comprise a whole antibody wherein the heavy chain is fused to human DNase I. Alternatively, the compound may comprise an Fab or F(ab')<sub>2</sub> fragment of an antibody wherein the truncated heavy chain (*i.e.* the Fd chain) is fused to human DNase I.

20 Preferably, the target-cell specific and the cytotoxic portion of the fusion compound of the invention separated by a linker sequence, for example to allow greater flexibility of the portions relative to one another.

More preferably, the linker sequence comprises a GG dipeptide.

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Most preferably the linker sequence is or comprises GG or GSGG.

Alternatively, the target-cell specific and the cytotoxic portion of the

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compound of the invention are separate moieties linked together by any of the conventional ways of cross-linking polypeptides, such as those generally described in O'Sullivan et al Anal. Biochem. (1979) 100, 100-108. For example, the antibody portion may be enriched with thiol groups and the enzyme portion reacted with a bifunctional agent capable of reacting with those thiol groups, for example the N-hydroxysuccinimide N-succinimidyl-3-(2iodoacetic acid (NHIA) or ester Amide and thioether bonds, for pyridyldithio)propionate (SPDP). example achieved with m-maleimidobenzoyl-N-hydroxysuccinimide ester, are generally more stable in vivo than disulphide bonds.

In a preferred embodiment of the first aspect of the invention, the compound comprises all or part of the amino acid sequence as shown in Figure 3(c) (i.e. an HMFG-1 light chain) together with all or part of an amino acid sequence selected from the group consisting of amino acid sequences as shown in Figures 5(d), 6(d), 7(b), 8(b), 9(b), 10(b), 11(b), 12(b), 13(d), 14(d), 15(d), 16(c), 17(d), 18(d) and 19(d) (i.e. an HMFG-1 heavy or Fd chain/DNase fusion).

Advantageously, the compound is a whole HMFG-1 antibody/human DNase I fusion compound comprising an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 7(b). Preferably, the compound is a tetrameric compound comprising two HMFG-1 light chains and two HMFG-1 heavy chain /DNase I fusions.

Conveniently, the compound comprises an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 14(d).

Preferably, the compound comprises one of the pairs of amino acid sequences defined above wherein the leader sequence of each amino acid (the first 19 amino acids of the sequences shown in each figure) is removed. It will be appreciated by persons skilled in the art that the compounds of the invention may also comprise variants of such amino acid sequences.

Suitably, the compound is a tetrameric compound comprising two HMFG1 light chains and two HMFG-1 Fd chain /DNase I fusions. More
10 preferably, the compound is a dimeric compound comprising one HMFG1 light chain and one HMFG-1 Fd chain /DNase I fusion.

A second aspect of the invention provides a nucleic acid molecule encoding a compound according to the first aspect of the invention, or a target cell-specific portion or cytotoxic portion thereof.

By "nucleic acid molecule" we include DNA, cDNA and mRNA molecules.

In a preferred embodiment of the second aspect of the invention, the nucleic acid molecule comprises all or part of the nucleotide sequence as shown in Figure 3(a or b) (i.e. encoding an HMFG-1 light chain) together with all or part of a nucleotide sequence selected from the group consisting of nucleotide sequences as shown in Figures 5(a, b and c), 6(a, b and c), 7(a), 8(a), 9(a), 10(a), 11(a), 12(a), 13(a, b and c), 14(a, b and c), 15(a, b and c), 16(a and b), 17(a, b and c), 18(a, b and c) and 19(a, b and c) (i.e. encoding an HMFG-1 heavy or Fd chain/DNase fusion).

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Advantageously, the nucleic acid molecule comprising a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 7(a).

5 Conveniently, the compound comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 14(c).

Alternatively, the nucleic acid molecule comprises nucleotide sequences that are degenerate sequences of those nucleotide sequences identified above (i.e. which encode the same amino acid sequence).

A further aspect of the present invention provides a method of making a compound according to the first aspect of the invention, said method comprising expressing one or more nucleic acid molecules according to the second aspect of the invention in a host cell and isolating the compound therefrom.

It is preferable that the two portions of the compound of the invention are produced as a fusion compound by recombinant DNA techniques, whereby a length of DNA comprises respective regions encoding the two portions of the compound of the invention either adjacent one another or separated by a region encoding a linker peptide which does not destroy the desired properties of the compound. The benefits in making the compound of the invention using recombinant DNA techniques are several fold. Firstly, it enables a high degree of precision with which the two portions of the compound can be joined together. Secondly, the construction of compounds which are "hetero-oligomeric" can be controlled by the expression of the different recombinant DNA molecules

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encoding each of the different type of subunit of the "hetero-oligomer" in the same host cell.

By "hetero-oligomer" we mean those compounds in which two or more different cell-specific portions are joined to either the same or to different subunits which are capable of oligomerisation. The expression, in the same host cell of two compounds, of A and B, each with different target cell specific portions but with a common second portion capable of oligomerisation will result in a mixed population of compounds. For example, if the common second portion is capable of dimerisation, three potential compounds will be produced: A<sub>2</sub>, AB and B<sub>2</sub>, in a ratio of 1:2:1, respectively.

The separation of the desired compound with each of the different cell specific portions, that is AB, can be achieved by two step affinity chromatography.

Application of the mixture of compounds to an affinity column specific for A will result in the binding of A<sub>2</sub> and AB. These compounds are eluted from this first column, and then applied to an affinity column specific for B. This will result in AB, but not A<sub>2</sub>, being bound to the column. Finally, the desired product AB, can be eluted.

Of course, the order in which the affinity columns are used is not important.

The same principle of separating those compounds with two or more different binding sites can be applied to the purification of the desired compounds from mixtures of other hetero-oligomers.

Conceivably, the two portions of the compound may overlap wholly or partly.

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Preferably, the compound is a multimeric compound such as a whole antibody/DNase fusion comprising two light chains and two heavy chains  $(H_2L_2)$ , a  $F(ab')_2$  fusion comprising two light chains and two truncated heavy chains  $(Fd_2L_2)$ , or a Fab fusion comprising one light chain and one truncated heavy chain (FdL).

The nucleic acid may be expressed in a suitable host to produce a polypeptide comprising the compound of the invention. Thus, the nucleic acid encoding the compound of the invention or a portion thereof may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the polypeptide of the invention. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter et al, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800 issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark et al, 4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to Itakura et al, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued 12 July 1988 to Toole, Jr. et al, 4,766,075 issued 23 August 1988 to Goeddel et al and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated herein by reference.

Where the compound of the invention is multimeric, the constituent chains

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may be encoded by a single nucleic acid molecule or separate nucleic acid molecule (expressed in a common host cell or in different host cells and assembled *in vitro*).

5 The nucleic acid encoding the compound of the invention or a portion thereof may be joined to a wide variety of other nucleic acid sequences for introduction into an appropriate host. The companion nucleic acid will depend upon the nature of the host, the manner of the introduction of the nucleic acid into the host, and whether episomal maintenance or integration is desired.

It will be appreciated that in order to prevent expression of the cytotoxic portion of the compound of the invention from killing the host cells in which it is expressed, it may be necessary to link the nucleic acid of the second aspect of the invention to a signal sequence capable of directing secretion of the expressed compound (or portion) out of the host cell. Signal sequences will be selected according to the type of host cell used. Exemplary signal sequences include the *ompA* signal sequence (for example, see Takahara *et al.*,1985, *J. Biol. Chem.* 260(5):2670-2674).

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Generally, the nucleic acid is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. If necessary, the nucleic acid may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by the desired host, although such controls are generally available in the expression vector. For example, the nucleic acid molecule encoding a compound of the invention may be linked to or comprise a Kozak consensus ribosome binding sequence (such as GCCGCCACC) to

enhance translation.

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The vector is then introduced into the host through standard techniques. Generally, not all of the hosts will be transformed by the vector. Therefore, it will be necessary to select for transformed host cells. One selection technique involves incorporating into the expression vector a nucleic acid sequence, with any necessary control elements, that codes for a selectable trait in the transformed cell, such as antibiotic resistance. Alternatively, the gene for such selectable trait can be on another vector,

Host cells that have been transformed by the recombinant nucleic acid of the invention are then cultured for a sufficient time and under appropriate conditions known to those skilled in the art in view of the teachings disclosed herein to permit the expression of the polypeptide, which can then be recovered.

which is used to co-transform the desired host cell.

Many expression systems are known, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells (for example COS-1, COS-7, CHO, NIH 3T3, NS0 and BHK cells) and insect cells (for example Drosophila, SF9 cells).

Those vectors that include a replicon such as a procaryotic replicon can also include an appropriate promoter such as a procaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

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A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention.

Typical procaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 (available from Biorad Laboratories, Richmond, CA, USA), pTrc99A and pKK223-3 (available from Pharmacia Piscataway, NJ, USA) and the pET system (T7 promoter, Novagen Ltd).

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers his3, trp1, leu2 and ura3. Plasmids pRS413-416 are Yeast Centromere plasmids (YCps).

Further useful vectors for transformation of yeast cells, such as *Pichia*, include the  $2\mu$  plasmid pYX243 (available from R and D Systems Limited) and the integrating vector pPICZ series (available from Invitrogen).

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion as described earlier, is treated with bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CN, USA.

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A desirable way to modify the nucleic acid encoding the compound of the invention or a portion thereof is to use the polymerase chain reaction as disclosed by Saiki et al (1988) Science 239, 487-491.

In this method the nucleic acid to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified nucleic acid. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

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Exemplary genera of yeast contemplated to be useful in the practice of the present invention are Pichia, Saccharomyces, Kluyveromyces, Candida, Torulopsis, Hansenula, Schizosaccharomyces, Citeromyces, Pachysolen, Debaromyces, Metschunikowia, Rhodosporidium, Leucosporidium, Debaromyces, Sporidiobolus, Endomycopsis, and the like. Preferred genera are those selected from the group consisting of Pichia, Saccharomyces, Kluyveromyces, Yarrowia and Hansenula. Examples of Saccharomyces are Saccharomyces cerevisiae, Saccharomyces italicus and Saccharomyces rouxii. Examples of Kluyveromyces are Kluyveromyces fragilis and Kluyveromyces lactis. Examples of Hansenula are Hansenula polymorpha, Hansenula anomala and Hansenula capsulata. Yarrowia lipolytica is an example of a suitable Yarrowia species.

Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 258 067 and WO 90/01063, all of which are incorporated herein by reference.

- Suitable promoters for *S. cerevisiae* include those associated with the *PGK1* gene, *GAL1* or *GAL10* genes, *CYC1*, *PHO5*, *TRP1*, *ADH1*, *ADH2*, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase, α-mating factor pheromone, a-mating factor pheromone, the *PRB1* promoter, the *GUT2* promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).
- The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, i.e. may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae AHD1* gene is preferred.

The present invention also relates to a host cell transformed with a polynucleotide vector construct of the present invention. The host cell can be either procaryotic or eukaryotic. Bacterial cells are preferred procaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research Laboratories Inc., Bethesda, MD, USA, and RR1 available from the

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American Type Culture Collection (ATCC) of Rockville, MD, USA (No ATCC 31343). Preferred eukaryotic host cells include yeast and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic cell line. Preferred eukaryotic host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CCL61, NIH Swiss mouse embryo cells NIH/3T3 available from the ATCC as CRL 1658 and monkey kidney-derived COS-1 cells available from the ATCC as CRL 1650 or WSØ cells.

- Transformation of appropriate cell hosts with a nucleic acid constructs of 10 the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen et al, Proc. Natl. Acad. Sci. USA, 69: 2110 (1972); and Sambrook et al, Molecular Cloning, A Laboratory Manual, Cold Spring Harbor 15 Laboratory, Cold Spring Harbor, NY (1989). Transformation of yeast cells is described in Sherman et al, Methods In Yeast Genetics, A Laboratory Manual, Cold Spring Harbor, NY (1986). The method of Beggs, Nature, 275: 104-109 (1978) is also useful. With regard to 20 vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc, Gaithersburg, MD 20877, USA.
- Successfully transformed cells, *i.e.* cells that contain a nucleic acid construct of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the

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polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern, J. Mol. Biol., 98: 503 (1975) or Berent et al, Biotech., 3: 208 (1985). Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

In addition to directly assaying for the presence of recombinant nucleic acid, successful transformation can be confirmed by well known immunological methods when the recombinant nucleic acid is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains the protein.

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Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

A third aspect of the invention provides a vector comprising a nucleic acid according to the second aspect of the invention.

A fourth aspect of the invention provides a host cell comprising a vector according to the third aspect of the invention.

Preferably, the host cell is a mammalian cell.

More preferably the host cell is NS0 or CHO.

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A fifth aspect of the invention provides a pharmaceutical composition comprising a compound according to the first aspect of the invention and a pharmaceutically acceptable carrier.

The compounds and compositions of the invention are administered in any suitable way, usually parenterally, for example intravenously, intraperitoneally or, preferably (for bladder cancer), intravesically (i.e. into the bladder), in standard sterile, non-pyrogenic formulations of diluents and carriers, for example isotonic saline (when administered intravenously).

A sixth aspect of the invention provides a compound according to the first aspect of the invention for use in medicine.

The compounds and compositions of the invention may be used to treat a patient with any disease involving a dysfunction of a population of cells expressing PEM, said compounds and compositions selectively targeting and destroying said population of cells within a patient. For example, said compounds and compositions may be used in the treatment of cancer, e.g. cancer of the breast, ovaries, lung, stomach, intestines, blood etc. Thus, anti-tumour cell antigen antibodies can be used to deliver a cytotoxic portion with endonuclease activity to a tumour cell. Antibodies that are internalised upon contact with the target antigen are used, such that the

cytotoxic portion enters the cytosol of the tumour cell, where it can trigger cell death.

In principle, the compounds and compositions of the invention may be used to treat any mammal, including pets such as dogs and cats and agriculturally important animals such as cows, horses, sheep and pigs.

Preferably, the patient is human.

- A seventh aspect of the invention provides the use of a compound according to first aspect of the invention in the preparation of a medicament for treating a mammal having said target cells to be destroyed.
- 15 Preferably, the medicament is for treating cancer, such as ovarian cancer.

A eighth aspect of the invention provides a method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to the first aspect of the invention to said mammal.

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In a preferred embodiment of the seventh and eighth aspects of the invention, the mammal is a human.

Preferably, the target cells to be destroyed are cancer cells. More preferably, the cancer cells are epithelial cancer cells, such as ovarian, gastric, colorectal and/or pancreatic cancer cells. Most preferably, the cancer cells are ovarian cancer cells.

The invention will now be described in detail with reference to the following figures and examples:

Figure 1 shows the complete coding sequence of human DNAse I.

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Figure 2 shows (A) the mature DNAse peptide I sequence used in the exemplary Ab-DNase and Fab-DNase constructs, and (B) a truncated DNAse peptide I sequence encoded by a nucleotide sequence comprising a Kozak sequence (underlined).

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Figure 3 shows (A) the nucleotide sequence encoding the humanised HMFG1 light chain including leader peptide, (B) the nucleotide sequence of (A) further comprising a Kozak sequence (underlined), (C) the amino acid sequence of the humanised HMFG1 light chain including leader peptide (shaded) and (D) the nucleotide sequence encoding the humanised HMFG1 heavy chain including leader peptide,

Figure 4 shows the linker and hinge-linker oligonucleotides used in (A) the whole antibody-DNase and (B) the Fd-DNase exemplary constructs.

Note, in Figure 4(A) a deletion of one or more codons between the HMFG1 hinge and the linker is represented as △G.

Figure 5 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS23 comprising a leader sequence (underlined) and a linker sequence (double-underlined). Figure 5(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

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Figure 6 shows (A), (B) and (C) shows the nucleotide sequences of Figure 5 (A), (B) and (C), respectively, further comprising an SV40 NLS (double underlined) (pAS27). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion comprising an SV40 NLS (double underlined).

Figure 7 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS34 (as used in 'Ab-DNase' in Example 2), comprising a leader sequence (underlined) and a linker sequence (double-underlined).

Figure 8 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS35, comprising a leader sequence (underlined) and a linker sequence (double-underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 9 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS36, comprising a leader sequence (underlined) and a linker sequence (double-underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 10 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS37, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined).

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Figure 11 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS38, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined). The lower case 'g' represents a silent mutation caused by PCR amplification.

Figure 12 shows (A) the nucleotide sequence and (B) the translated amino acid sequence of an exemplary HMFG-1 heavy chain/DNase I fusion pAS39, comprising a leader sequence (underlined), a linker sequence (double-underlined) and an NLS sequence (triple underlined). The lower case 'c' represents a silent mutation caused by PCR amplification.

Figure 13 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS101 comprising a short leader sequence (underlined) and a linker sequence (double-underlined). Figure 13(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

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Figure 14 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS102 comprising a leader sequence (underlined) and a hybrid hinge + linker sequence (double-underlined). Figure 14(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined) (construct designated pAS302 in Example 2). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

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Figure 15 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS103 comprising a leader sequence (underlined) and a hybrid hinge + short linker sequence (double-underlined). Figure 15(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

Figure 16 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS104 comprising a leader sequence (underlined) and a hybrid hinge + mutated short linker sequence (double-underlined). Figure (C) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion. Mutations (compared to pAS103) at positions 775 and 924 are shaded.

Figure 17 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS105 comprising a leader sequence (underlined), a short linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 17(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

Figure 18 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS106 comprising a leader sequence (underlined), a hybrid hinge + linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 18(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1

Fd/DNase I fusion.

Figure 19 shows nucleotide sequences (A and B) encoding a humanised HMFG-1 Fd/DNase I fusion pAS107 comprising a leader sequence (underlined), a hybrid hinge + short linker sequence (double-underlined) and an NLS sequence (triple underlined). Figure 19(C) shows the nucleotide sequence of (B) further comprising a Kozak sequence (underlined). Figure (D) shows the amino acid sequence of a humanised HMFG-1 Fd/DNase I fusion.

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Figure 20 shows a schematic diagram of the pEE6 expression vector used in the exemplary constructs.

Figure 21 shows autoradiographs from immuno-precipitation experiments with metabolically labelled transient transfectants:

### GEL A

Lane 1 shows the precipitation of supernatant from mock-transfected cells.

Lane 2 is from cells transfected with hHMFG-1 (construct 6) giving expected molecular weights of about 51.2 and 26.4 kDa for the heavy and light chains, respectively.

Lane 3 shows construct 34 antibody construct which has human DNase I fused to the C-terminus of the heavy chain gene. As expected, the size of the heavy chain gene has increased to about 80.7 kDa.

Samples from whole antibody DNase I constructs 35, 36 and 39 were run on the gel (Lanes 4 to 6) but were not sufficiently well

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expressed to be visible, in this experiment.

the 3'-end of the fusion mRNA).

In subsequent experiments using this method, construct 39 was detectable but weak, and constructs 35 and 36 were detectable but very weak. Constructs 37 and 38 have not been tested in this assay system.

Lanes 8 to 10 are fusion of humanised HMFG1 F(ab')<sub>2</sub> with human DNase I (constructs 41, 23 and 102, respectively). F(ab')<sub>2</sub> alone was included in this set of experiments (lane 7, construct 41) but did not express, this was included in later experiments (see gels C and D). In addition to the light chain (about 26.4 kDa) and the Fd-DNAse I fusion (about 56.6 kDa), a third major band is observed at around 40 kDa. Interestingly, this band is observed in the humanised HMFG-1 fusions but not in the antibody alone. Since an anti-F(ab')<sub>2</sub> antibody was used for immuno-precipitation, it is unlikely that this can be proteolysis between immunoglobulin and DNase I sequence. It probably represents a population of polypeptide produced by premature transcriptional termination (due to DNase I sequence in

### 20 **GEL B**

This is the non-reducing gel counterpart to gel A, described above. Lane 1 is the mock-transfected control cells and lanes 2 and 3 are from the cells transfected with humanised HMFG1 alone (construct 6) and the humanised HMFG-1 fused at the C-terminus to human DNase I, respectively. As before, lanes 4 to 6 are from cell supernatants from cells transfected with constructs 35, 36 and 39. The gel shows that both the whole antibody and the antibody-DNase I fusion are assembled, with the DNase fusion giving a higher

molecular weight compared to the antibody alone.

Figure 22 shows a typical standard curve used to determine the concentration of PDTRP-binding material in the supernatants of transiently transfected L761h cells. Each point on the curve has been determined twice.

Figure 23 shows typical standard curves used to determine the concentration of bovine DNAse I.

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Figure 24 shows corrected DNase I activity in transiently expressed humanised HMFG1 whole antibody-human DNAse I fusions (*i.e.* pAS34, pAS34, pAS35 and pAS6[control]).

Figure 25 shows the corrected DNAse I activity in transiently expressed humanised HMFG1 F(ab')<sub>2</sub>-human DNase I fusions (i.e. pAS101, pAS102, pAS103 and pAS41[control]).

Figure 26 shows results of the cytotoxicity assay.

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Figure 27 shows the % of MCF7 cells killed after incubation with the exemplary constructs.

Figure 28 shows a schematic diagram of (A) Ab-DNase and (B) Fab-DNase.

Figure 29 shows a schematic diagram of vector pAS34K encoding Ab-DNase (i.e. pAS34 as shown in Figure 7b plus Kozak sequence). WO 01/74905 PCT/GB01/01324

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Figure 30 shows a schematic diagram of vector pAS302 encoding Fab-DNase.

5 Figure 31 shows (A) the elution profile from Protein-L column and (B) size exclusion chromatogram for Fab-DNase.

Figure 32 shows (A) the elution profile from Protein-A column and (B) size exclusion chromatogram for Ab-DNase.

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Figure 33 shows the SDS-PAGE stained gels for (A) Ab-DNase and (B) Fab-DNase.

Figure 34 shows (A) standard curve for bovine DNase concentration AND

(B) DNase activity measurements at 3 hours and 6 hours.

Figure 35 shows (A) PEM expression on OVCAR 3 and A375 cells, as measured by ELISA using hHMFG-1 and AD-DNase antibodies, and (B) cytotoxicity measurements.

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#### **EXAMPLES**

#### Example 1

## 5 (A) Mammalian expression of humanised HMFG1-DNase constructs

The human HMFG1 light and heavy chain (with or without engineering a fusion to human DNase I), were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NSO cells (see 10 figure 20). The vector system was originally developed by Celltech Ltd (UK) and is now owned by al-Lonza (see Young & Owens, 1994, J. Immunol. Meth. 168:149-165). The vector consists of two human cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal 15 (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. Propagation in E.coli can be selected for by the presence on an ampicillin resistance gene (not shown in Fig 20). The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NS0 transfectomas to be selected by 20 growth in glutamine free media, since NSO cells are GS and cannot otherwise grow in glutamine free media.

Exemplary humanized HMFG1-DNAse I fusion constructs of the invention are detailed in figures 5 to 19.

(B) Immuno-precipitation of metabolically labelled transient transfectants

CHO-L761h cells (Cockett et al., 1990, Nuc. Acids Res. 19:319-325)

were transfected, according to the modification of Gorman et al, 1985), with expression vectors containing either whole HMFG1 antibody or  $F(ab')_2$  fragment of the antibody along with the various fusion constructs of their respective heavy chains and human DNase I. The cells were then incubated with either 50  $\mu$ Ci <sup>35</sup>S methione for 72 h in methionine-free medium. Secreted product was precipitated with a rabbit anti-human  $F(ab')_2$  antibody bound to protein A Sepharose. Bound material was eluted in either reducing or non-reducing SDS-PAGE loading buffer and run on gels. The autoradiographs (see Figure 21) above were generated from those gels after drying them.

### (C) Estimation of the efficiency of DNase constructs in supernatants

#### Introduction

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This set of experiments was designed to standardise the amount of construct in a given DNase I activity assay and to allow us to comment on the amount of activity a particular construct possesses. Given that the antibody-DNase I fusions are so different to the F(ab')<sub>2</sub>-DNase I fusions it is best not to compare the two groups. Once we have purified the protein, we will have a better idea of the exact molecular configuration of all species. Then, and only then, will it be sensible to compare amongst groups.

### 25 Determination of concentration of constructs

The concentration of constructs in supernatants from transiently transfected L761H cells was determined in a PDTRP-binding ELISA. To

each well of a Maxisorb 96-well ELISA plate (Nunc) was added 100  $\mu$ l of carbonate buffer containing 100 ng of recombinant GST-(PDTRP)<sub>7</sub> fusion protein (Gendler *et al.*, 1990, *J. Mol. Biol.* 265:15286-93). After overnight binding at 4°C, the plate was washed three times in PBS-Tween (*i.e.* PBS containing 0.05% Tween-20). The plate was then blocked with three 3-minute washes of PBS-Tween containing 1% BSA.

For each construct,  $100 \mu l$  of supernatant was added to a well on the plate. In addition, hHMFG-1 of known concentration was serially diluted down the plate using doubling dilutions in  $100 \mu l$  of PBS-Tween per well. The plate was incubated for a further 1 h at  $30^{\circ}$ C, then 200 ng of MC135 antihuman kappa light chain antibody (binding site) in  $100 \mu l$  of PBS-Tween was added to each well for 1 h at  $30^{\circ}$ C. After three 3-minute washes in PBS-Tween,  $100 \mu l$  of anti-mouse IgG-peroxidase conjugate (Jackson 315-035-045), diluted 1:2000 in PBS-Tween, was added to each well and incubated for 1 h at  $30^{\circ}$ C. Following a final set of three 3-minute washes in PBS-Tween,  $100 \mu l$  of TMB substrate (Sigma) was added to each well of the plate and, after a colour developed, the optical density at 630 nm of the solution in each well of the plate was determined.

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Results

(see Figure 22)

## 25 (D) Corrected bovine DNase I standard curves and DNase assay

DNase activity was determined using a modification of the methyl green-DNA complex degradation method (Sinicropi et al., 1994, Analyt. 5

Biochem. 222:351-358). Briefly, a 1:1 solution of the assay buffer and methyl green-salmon sperm DNA complex was mixed together to give a total volume of 0.2 ml. To this, 0.1 ml of tissue culture supernatant from transiently transfected CHO-L761h cells was added and the mixture incubated at 37°C. DNA cleavage by DNase results in a reduction in absorbance at 620 nm. Figure 23 shows a standard curve produced with various concentrations of bovine DNase I over a number a time point.

Figures 24 and 25 show DNAse activity for the whole HMFG1 antibodyand  $F(ab')_2$ - DNase fusions, respectively.

#### (E) Cytotoxicity of DNAse constructs

#### Method

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DNase constructs were transfected into CHO L761h cells using a calcium phosphate co-precipitation method (Gorman et al., 1985, In: DNA cloning (2nd edition), Glover A(ed.), Academic Press, NY, 163-188). Included in the experiment were negative controls, consisting of cells transfected with TE buffer alone or with TE buffer and pEE6 expression vector. In addition to these controls, vectors that express hHMFG-1 (pAS6) and F(ab')<sub>2</sub> of hHMFG1 (both with specificity for PEM but without DNase I) were included.

The supernatant from these cells was harvested after 72 h of expression, followed by centrifugation to remove dead cells. MCF-7 cells were incubated for 1 h at 37°C with an aliquot of each of these supernatants. The amount of cellular lactate dehydrogenase (LDH) released from the

MCF-7 cells due to the cytotoxicity of the supernatant was determined using the CytoTox96 cytotoxic assay kit (Promega). Total lysis ('total LDH') was determined by measuring the target cell maximum LDH release using the kits lysis solution. The percentage of cells killed was then calculated as the proportion of the LDH released to the total LDH released. For each construct, the cytotoxicity assay was performed in quadruplicate, except for assay of pAS38 and 39, which were performed in triplicate. The values of LDH release for each construct were compared against either F(ab')<sub>2</sub> or whole antibody, or each other, using a one-tailed t-test in Excel.

#### Results

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Figures 26 and 27 shows that there is negligible cell killing with either pAS6 (HMFG1 alone) or with pAS41 (F(ab')<sub>2</sub> alone). All of the hHMFG1 F(ab')<sub>2</sub>-DNase I constructs kill significantly more cells than the F(ab')<sub>2</sub> fragment alone (p<0.00193) and all of the antibody-DNase I constructs kill significantly more cells than antibody alone (p<0.00783), except for perhaps pAS34 (p<0.021).

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# (F) Use of the DNase-I/huHMFG-1 Fab fusion protein in the treatment of ovarian cancer

Patients diagnosed with ovarian cancer are treated by intravenous injection of the DNaseI/huHMFG-1 Fab fusion protein. Typically, a dose of between 1 to 100 mg will be administered weekly.

Therapeutic response is measured by the normal clinical procedures that

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are well known in the art, for example radio-imaging methods.

#### Example 2

## 5 (A) Mammalian expression of humanised HMFG-1 / DNase constructs

In a second series of experiments, two further humanised HMFG-1/Dnase constructs were expressed in mammalian cells. The first construct encoded a fusion protein a complete hHMFG-1 antibody fused with human DNase, designated 'Ad-DNase'. The second construct encoded a fusion protein a Fab fragment of the hHMFG-1 antibody fused with human DNase, designated 'Fab-DNase'. Ad-Dnase and Fab-DNase are shown schematically in Figure 28.

Ad-DNase comprises an HMFG-1 light chain as shown in Figure 3(c) and an HMFG-1 heavy chain/DNase fusion as shown in Figure 7(b).

Fab-DNase comprises an HMFG-1 light chain as shown in Figure 3(c) and an HMFG-1 Fd chain/DNase fusion as shown in Figure 14(d).

The human HMFG1 heavy and light chain constructs were cloned into the pEE6 expression vector system for expression in mammalian CHO or myeloid NS0 cells, as described in Section (A) of Example 1. This vector consists of two human cytomegalovirus promoters (hCMV) for both the heavy and light chain genes. Each transcription unit is completed by the poly-adenylation signal (pA) with an optional immunoglobulin terminator sequence (Ig term.) located between the heavy and light chain transcription units. The vectors also comprise a 5'-UT Kozak sequence

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(to enhance translation of the mRNA) and an ATG initiator codon upstream of both heavy and light chains.

The vectors encoding Ad-Dnase and Fab-DNase, designated pAS34K and pAS 302 respectively, are shown schematically in Figure 32.

Propagation in *E.coli* can be selected for by the presence on an ampicillin resistance gene. The inclusion of a glutamine synthetase gene (GS) in the vector allows the stable NSO transfectomas to be selected by growth in glutamine free media, since NSO cells are GS and cannot otherwise grow in glutamine free media.

These plasmids were co-transfected with a vector containing a neomycin resistance gene into CHO cells. Stable cell lines were generated for each of the constructs.

Clones were selected that expressed DNase activity and antigen (PEM)-binding activity.

## 20 (B) Purification of hHMFG-1/DNase constructs

The cells were routinely grown in:

	DMEM (Gibco 10938-025)	500 ml
25	Non essential amino acids (Sigma M7145)	5 ml
	Sodium pyruvate (Sigma S8636)	5 ml
	Glutamine (G7513)	5 ml
	Heat inactivated foetal calf serum	50 ml

Incubation was carried out at 37°C in 5% CO<sub>2</sub>.

For production of the Ab-DNase fusion protein, W70 cells (CHO cells transfected with pAS34K) were maintained in flats and grown to confluency in T175 flasks. Each T175 flask was split between two 850 cm<sup>2</sup> roller bottles containing 100 ml of the aforementioned growth media. Each roller bottle was gassed with an 95% air 5% CO<sub>2</sub> mix for 1 minute and then sealed. They were rolled at a rate of 0.5 rpm and were gassed every other day as described earlier until the cultures were 10 confluent. At this stage the medium was removed and 200 ml of harvest medium was replaced on the culture. This was the same medium but contained 2 mM sodium butyrate (with or without 10% heat inactivated FCS). The cells were then grown for a further 3-4 days before they were harvested. The medium was collected from the cells and dead cells were 15 removed from the medium by centrifugation at 5000 rpm for 30 mins at 4°C. The spun medium (supernatant) was then filtered through a 0.2 micron filter unit, prior to applying to the affinity chromatography column.

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The Fab-DNase fusion product was then purified by affinity chromatography using a Protein-L column (Protein L agarose, P3351 from Sigma Co, Poole, Dorset, UK), as follows:

- Wash 1 ml of settled protein L agarose (P3351) with at least 5 volumes of phosphate buffered saline (PBS: 10 mM phosphate buffered saline, pH 7.4).
  - 2. Dilute 1 ml supernatant with 9 ml PBS.

- 3. Mix diluted supernatant with protein-L agarose and incubate with gentle end over end mixing for 1 hour at room temperature.
- 4. Pack the slurry in a column and drain.
- 5. Wash away unbound proteins with 10-15 column volumes of PBS.
- Elute bound protein with ml elution buffer (0.1 M glycine, pH 2.0, or 0.2 M citrate buffer, pH2.8).
  - 7. Neutralise eluted material with Tris-base to achieve pH 7.5.

Figure 31(a) shows the elution profile of the Fab-DNase from the Protein-10 L column when eluted with 0.1 M glycine, pH 2.0.

Following purification, Fab-DNase was analysed by analytical size-exclusion chromatography on a Superdex-200 column.

Figure 31(b) shows the size-exclusion chromatogram obtained for the Fab-DNase.

The Ab-DNase fusion product was purified by affinity chromatography using a Protein-A sepharose column, as follows:

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- 25 ml of protein A sepharose fast flow resin (Amersham Pharmacia Biotech) in an XK26 column (Amersham Pharmacia Biotech) was equilibrated in 0.1M glycine, pH8.8, 0.5M NaCl.
- Approximately 2 litres of sterile-filtered supernatant from cell line W70 (CHO cell line making 34K) was passed the column overnight at a low flow rate (1-2 ml/min).
  - 3. The column was then washed down to base-line and was reequilibrated in 0.15M disodium hydrogen phosphate, pH9.0 and the

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bound 34K was eluted by running a gradient between this buffer (A) and a low pH buffer (B) which consisted of 0.1M citric acid, pH2.0, supplemented to 2 mM calcium chloride and 2 mM magnesium sulphate. The gradient was run over 100 ml at a flow rate of 4 ml/min and a further 50 ml of buffer B was run over the column at the completion of the gradient, also at 4 ml/min.

- 4. During the 100 ml gradient and the last 50 ml of buffer A fractions were collected. The peak fractions were identified and pooled and dialysed against 4 litres of 25 mM Hepes, pH7.5, 0.2 M NaCl, 1mM calcium chloride and 1mM magnesium sulphate. Dialysis was performed overnight at 4C.
- 5. The dialysate was concentrated on Centricon spin concentrators to a final concentration of 6-13 mg/ml. The concentration was determined by dividing by its extinction coefficient of 1.558 (calculated from the known sequence).

Figure 32(a) shows the elution profile of the Ab-DNase from the Protein-L column when eluted with a gradient of 0.15 M Na<sub>2</sub>HPO<sub>4</sub>, pH 9.0 to 0.1 M citric acid, pH 2.0 containing 2mM each of CaCl<sub>2</sub> and MgCl<sub>2</sub>.

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Figure 32(b) shows the size-exclusion chromatogram obtained for the Ab-DNase.

## (C) Determination of concentration of fusion proteins

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Prior to measuring DNase activity of the purified fusion proteins (see Section (E) below), the concentration of the proteins was determined by ELISA, as follows (see also Section (C) of Example 1).

#### Materials

- 1. 96 Well ELISA plates (Nunc F96 Maxisorp Cat No. 442404).
- 5 2. Bovine serum albumin (Sigma A-9647).
  - 3. Coating buffer (Na2CO3 1.59 g/l, NaHC03 2.93 g/l, NaN3 0.2 g/l, pH9.6.
  - 4. GST-MUC1-7TR antigen (1.5 mg/ml).
  - 5. Anti-human kappa light-chain antibody GD12 (0.2 mg/ml, Binding Site, MC135).
    - 6. Peroxidase-conjugated rabbit anti-mouse IgG (Jackson, 315-035-045).
    - 7. TMB- substrate buffer (Sigma P-4417).
    - 8. Tween 20 (Sigma P7949).
- 9. Purified humanised HMFG1 (1.4 mg/ml).

#### Method

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Note all washes in this protocol consist of 3 x 3 min washes in PBS buffer (note: all PBS buffer contained 0.05 % Tween) and the plate was incubated in a lunch box containing moist tissue paper.

- 1. Coat 100 ng of antigen/100  $\mu$ l coating buffer/well overnight at 4°C.
- Wash the plate and block each well with 100 μl of PBS containing 0.05 % Tween, and 1% BSA for 1 h at 30°C. Wash plate afterwards.
  - 3. A standard curve of humanised HMFG1 should be prepared

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down the plate using doubling dilutions. Make each dilution in  $100 \ \mu l$  PBS buffer and for the highest concentration in the curve use  $1000 \ ng$  of antibody.

- 4. Incubate the plate for 2 h at 30°C, wash, and add 100 μl PBS containing 200 ng of the anti-human Kappa light chain antibody to each well of the plate. Incubate for a further 1 h at 30°C and then wash the plate.
- Add 100 μl PBS containing the rabbit anti-mouse IgG-peroxidase conjugate (diluted 1:2000) to each well of the plate and incubate for 30 min at 30°C. Wash the plate and add 100 μl TMB- substrate-buffer to each well of the plate and allow the reaction to proceed in the dark at room temperature. When the blue colour has developed, read the plate at a wavelength of 630 nm.

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#### (D) SDS-PAGE

Following purification of Ab-DNase and Fab-DNase, the fusion proteins were analysed by SDS-PAGE under non-reducing and reducing conditions, as described in Section (B) of Example 1.

In brief, affinity-purified material was used. In the case of the Ab-DNase fusion protein, this was from a sample dialysed and concentrated (as described in the protein A protocol above). In the case of the Fab-DNase, this was unconcentrated protein directly eluted from the protein L affinity column. 15 ul of the Fab-DNase protein-L eluate was mixed with 5 ul of either reducing or non-reducing loading buffer whereas 2 ul of the Ab-DNase protein A eluate (dialysed and concentrated) was mixed with 5 ul

of either reducing or non-reducing buffer. Both samples were boiled for 5 minutes and were loaded onto the gel. The gels were stained with Coomassie Brilliant Blue stain. The cells were not labelled with 35S-methionine (as in Example 1).

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The SDS-PAGE autoradiograph for Ab-DNase is shown in Figure 33(a). Under reducing conditions, Ab-DNase produces a band of about 80 kDa, which corresponds to the expected size of the heavy chain-DNase fusion product (see lane 3). A further band of about 50 kDa is also observed, which is approximately the same molecular weight as the hHMFG-1 heavy chain (see lane 4).

The SDS-PAGE autoradiograph for Fab-DNase is shown in Figure 33(b). Under reducing conditions, Fab-DNase produces a band of about 55-60 kDa, which corresponds to the expected size of Fab-DNase (see lane 3). Under non-reducing conditions, a band of about 80-85 kDa is observed, which is the approximate molecular weight of Fab-DNase rather than F(ab')<sub>2</sub>-DNase (see lane 4). Thus, the Fab-DNase appears to exist as a dimer of the hHMFG-1 light chains and the hHMFG-1 heavy chain/human DNase fusion, not a tetrameric F(ab')<sub>2</sub>-DNase.

#### (E) Measurement of DNase activity of hHMFG-1/DNase constructs

DNase activity of the two fusion proteins was determined as described in Section (D) of Example 1. In brief, 0.1 ml of the purified protein was added to a 1:1 solution of assay buffer and methyl green-salmon sperm DNA complex, and the mixture incubated at 37°C. A reduction in absorbance at 620 nm is indicative of DNA activity.

A standard curve produced using bovine DNase I is shown in Figure 34(a).

Figure 34(b) shows the DNase activity of the Fab-DNase and Ab-DNase fusion proteins 3 h and 6 h after being added to the DNA, compared to a positive control of bovine DNase and a negative control of Fab only. Clearly, the DNase activity of the Fab-DNase and Ab-DNase fusion proteins is comparable to that of the bovine DNase positive control.

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# (F) Cytotoxicity of DNase activity of hHMFG-1/DNase constructs

Cytotoxocity of the Fab-DNase and Ab-DNase fusion proteins was analysed using two tumour cell lines, the human malignant melanoma cell line A375 and the human ovarian adenocarcinoma cell line OVCAR 3.

An initial cell-based ELISA was performed using hHMFG-1 antibodies to determine the level of expression of PEM (the MUC1 gene product) on these cells.

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## Cell-based PEM ELISA assay protocol

#### Materials and methods

- 25 1. Phosphate buffered saline tablets (Sigma P-4417)
  - 2. 50% glutaraldehyde solution (BDH UN2810 Prod. 2868240)
  - 3. sodium azide (Sigma S-8032)
  - 4. Nunclon 96 well tissue culture plate (Nunc D167008)

- 5. BSA (Sigma A-9647)
- OVCAR-3 ovarian cancer cells, A375 melanoma cancer cells both from ATCC
- 7. TMB substrate buffer (Sigma P-4417)
- 5 8. Tween 20 (Sigma P7949)
  - 9. Purified humanised HMFG1 (1 mg/ml from ICRF)
  - 10. RPMI 1640 media (Gibco 21875-034)

#### Protocol

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- 1. The OVCAR-3 and A375 cells were grown in RPMI containing 20% and 10% FCS respectively at 37°C in 5% CO2 in a 96 well tissue culture plate, seeded at 106 cells/ml with 0.1 ml/well.
- 2. Excess media was removed and the plate was fixed with 0.05% glutaraldehyde in water for 1 hour at room temperature.
- Excess glutaraldehyde/water solution was removed and the plates were washed three times with PBS containing 0.05% Tween 20.
   The plate was stored at 4°C until required in PBS with 0.02% sodium azide).
- 4. To use the plate, the plate was then washed with three washes of PBS containing 0.05% Tween 20, and the wells were blocked with 0.1 ml 5% BSA in PBS containing 0.05% Tween 20. The wells were blocked for 1 hour at 30°C.
- 5. They washed three times as described before. Serial dilutions of hHMFG1 were plated out on the wells from a maximum concentration of 2 μg/ml downward. Dilutions of constructs were also similarly plated onto the fixed cells. All dilutions were prepared in PBS containing 0.05% Tween 20.

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- 6. The proteins were incubated with the fixed cells for 1 hour at 30°C and were again washed three times as described above.
- Anti-human IgG-Fc peroxidase conjugate antibody (Jackson 209-035-103) was diluted to 1:2000 in PBS containing 0.05% Tween
   This was incubated at 30°C for 30 minutes.
- 8. Once again the cells were washed as described as before. Then 0.1 ml TMB substrate was put in each well and the colour was developed at room temperature and the absorbance at 655 nm was determined.

For comparison, an additional ELISA using Ab-DNase was performed with the OVCAR 3 cells.

Antigen-bound hHMFG-1 and Ab-DNase was detected by a peroxidaseconjugated anti-human Fc antibody.

The results of the ELISA are shown in Figure 35, indicating that the OVCAR 3 cell line expresses high levels of PEM (as measured by both hHMFG-1 and Ab-DNase) while the A375 cell line expresses low levels of PEM (and hence can be used as a negative control in cytotoxicity experiments).

Cytotoxicity was measured using an LDH release assay, as described in Section (E) of Example 1. In brief, 10<sup>5</sup> cells per well of the A375 and OVCAR 3 cell lines were plated in a 96-well plate and grown for 24 hours. Fifteen microlitres of the purified fusion proteins (containing 200 ng of Ab-DNase or 100 ng of Fab-DNase) were added to the cells and incubated for 48 hours at 37°C. A negative control group of each cell

type was treated with 200 ng of the hHMFG-1 antibody (i.e. not fused to DNase).

Following the incubation period, 50  $\mu$ l of the supernatant was removed and incubated with 50  $\mu$ l of tetrazolium-containing substrate buffer for 30 minutes at 22°C. The reaction was stopped with stop buffer (Promega) and the absorbance of the reaction mixture at 490 nm measured.

Both Fab-DNase and Ab-DNase fusions show cell killing of OVCAR 3

10 cells as compared to the negative control hHMFG-1 treated cells. In contrast, killing of A375 cells by DNase fusions is negligible, consistent with negligible binding of the fusions to these cells.

#### **CLAIMS**

- 1. A compound comprising a target cell-specific portion and a cytotoxic portion characterised in that:
  - (i) the target cell-specific portion comprises an humanised monoclonal antibody having specificity for polymorphic epithelial mucin (PEM), or an antigen binding fragment thereof; and
  - (ii) the cytotoxic portion has endonucleolytic activity.
- A compound according to Claim 1 wherein the target cell-specific portion comprises an humanised HMFG-1 antibody or an antigen binding fragment thereof.
- 3. A compound according to Claim 2 wherein the target cell-specific portion is an humanised HMFG-1 antibody.
- 4. A compound according to Claim 1 or 2 wherein the target cell-specific portion comprises an antigen binding fragment of the humanised antibody selected from the group consisting of Fab-like molecules, such as Fab and F(ab')<sub>2</sub>, Fv molecules, disulphide-linked Fv molecules, ScFv molecules and single domain antibodies (dAbs).
- 5. A compound according to Claim 4 wherein the target cell-specific portion comprises a Fab molecule.
- 6. A compound according to Claim 4 wherein the target cell-specific

portion comprises a F(ab')<sub>2</sub> molecule.

- 7. A compound according to Claim 1 wherein the target cell-specific portion comprises an amino acid sequence encoded by at least part of one or both of the nucleotide sequences of Figure 3(a) and (d).
- 8. A compound according to Claim 7 wherein the target cell-specific portion comprises an amino acid sequence encoded by the nucleotide sequence of Figure 3(a) and an amino acid sequence encoded by the nucleotide sequence of Figure 3(d).
- 9. A compound according to any one of Claims 1 to 8 wherein the cytotoxic portion has DNA endonucleolytic activity.
- 10. A compound according to Claim 9 wherein the cytotoxic portion is at least the catalytically active portion of a DNA endonuclease.
- 11. A compound according to Claim 10 wherein the endonuclease is a mammalian deoxyribonuclease I.
  - 12. A compound according to Claim 11 wherein the endonuclease is a human deoxyribonuclease I.
- 13. A compound according to Claim 1 wherein the endonuclease is a restriction endonuclease.
- 14. A compound according to Claim 10 wherein the cytotoxic portion comprises the amino acid sequence shown in Figure 2(a) or (b).

- 15. A compound according to any one of Claims 1 to 14 wherein a nuclear localization signal is incorporated.
- 16. A compound according to Claim 15 wherein the nuclear localization signal comprises the sequence PKKKRKV.
- 17. A compound according to any one of Claims 1 to 16 wherein the target cell-specific portion and the cytotoxic portion are fused.
- 18. A compound according to Claim 17 wherein the target cell-specific portion and the cytotoxic portion are separated by a linker sequence.
- A compound according to Claim 18 wherein the linker sequence is or comprises GG or GSGG.
- 20. A compound according to any one of Claims 1 to 19 wherein the compound comprises all or part of the amino acid sequence as shown in Figure 3(c) together with all or part of an amino acid sequence selected from the group consisting of amino acid sequences as shown in Figures 5(d), 6(d), 7(b), 8(b), 9(b), 10(b), 11(b), 12(b), 13(d), 14(d), 15(d), 16(c), 17(d), 18(d) and 19(d).
- 21. A compound according to Claim 20 wherein the compound comprises an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 7(b).
- 22. A compound according to Claim 20 wherein the compound comprises

an amino acid sequence as shown in Figure 3(c) and an amino acid sequence as shown in Figure 14(d).

- 23. A nucleic acid molecule encoding a compound as defined in any one of Claims 1 to 22.
- 24. A nucleic acid molecule according to Claim 23 wherein the molecule comprises all or part of the nucleotide sequence as shown in Figure 3(a or b) together with all or part of a nucleotide sequence selected from the group consisting of nucleotide sequences as shown in Figures 5(a, b and c), 6(a, b and c), 7(a), 8(a), 9(a), 10(a), 11(a), 12(a), 13(a, b and c), 14(a, b and c), 15(a, b and c), 16(a and b), 17(a, b and c), 18(a, b and c) and 19(a, b and c).
- 25. A nucleic acid molecule according to Claim 24 wherein the molecule comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 7(a).
- 25. A nucleic acid molecule according to Claim 24 wherein the molecule comprises a nucleotide sequence as shown in Figure 3(b) and a nucleotide sequence as shown in Figure 14(c).
- 26. A nucleic acid molecule according to any one of Claims 23 to 25 wherein the molecule further comprises a Kozak consensus ribosome-binding site.
- 27. A vector comprising a nucleic acid molecule according to any one of Claims 23 to 26.

- 28. A host cell comprising a vector according to Claim 27.
- 29. A pharmaceutical composition comprising a compound according to any one of Claims 1 to 22 and a pharmaceutically acceptable carrier.
- 30. A compound according to any one of Claims 1 to 22 for use in medicine.
- 31. Use of a compound according to any one of Claims 1 to 22 in the preparation of a medicament for treating a mammal having said target cells to be destroyed.
- 32. A method of treating a mammal having target cells to be destroyed, the method comprising administering a compound according to any one of Claims 1 to 22 to said mammal.
- 33. A use according to Claim 31 or a method according to Claim 32 wherein the mammal is a human.
- 34. A use according to Claim 31 or a method according to Claim 32 wherein the target cells to be destroyed are cancer cells.
- 35. A use or a method according to Claim 34 wherein the cancer cells are epithelial cancer cells.
- 36. A use or a method according to Claim 35 wherein the cancer cells are ovarian, gastric, colorectal and/or pancreatic cancer cells.

- 37. A use or a method according to Claim 36 wherein the cancer cells are ovarian cancer cells.
- 38. A compound substantially as described herein, preferably with reference to one or more of the accompanying figures.

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## Human DNase I

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            Recombinant human DNase I reduces the viscosity of cystic fibrosis
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            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
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# 2/113 Human DNase I construct

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            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
            Recombinant human DNase I reduces the viscosity of cystic fibrosis
  TITLE
  JOURNAL
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
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# 3/113

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                         260 C
                                  251 G
                                            170 T
BASE COUNT
                177 A
ORIGIN
        1 GCCGCCACCA TGAGGGGCAT GAAGCTGCTG GGGGCGCTGC TGGCACTGGC GGCCCTACTG
       61 CAGGGGGCCG TGTCCCTGAA GATCGCAGCC TTCAACATCC AGACATTTGG GGAGACCAAG
      121 ATGTCCAATG CCACCCTCGT CAGCTACATT GTGCAGATCC TGAGCCGCTA CGACATCGCC
      181 CTGGTCCAGG AGGTCAGAGA CAGCCACCTG ACTGCCGTGG GGAAGCTGCT GGACAACCTC
      241 AATCAGGACG CACCAGACAC CTATCACTAC GTGGTCAGTG AGCCACTGGG ACGGAACAGC
      301 TATAAGGAGC GCTACCTGTT CGTGTACAGG CCTGACCAGG TGTCTGCGGT GGACAGCTAC
      361 TACTACGATG ATGGCTGCGA GCCCTGCGGG AACGACACCT TCAACCGAGA GCCAGCCATT
      421 GTCAGGTTCT TCTCCCGGTT CACAGAGGTC AGGGAGTTTG CCATTGTTCC CCTGCATGCG
      481 GCCCCGGGGG ACGCAGTAGC CGAGATCGAC GCTCTCTATG ACGTCTACCT GGATGTCCAA
      541 GAGAAATGGG GCTTGGAGGA CGTCATGTTG ATGGGCGACT TCAATGCGGG CTGCAGCTAT
      601 GTGAGACCCT CCCAGTGGTC ATCCATCCGC CTGTGGACAA GCCCCACCTT CCAGTGGCTG
      661 ATCCCCGACA GCGCTGACAC CACAGCTACA CCCACGCACT GTGCCTATGA CAGGATCGTG
      721 GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT GTTCCCGACT CGGCTCTTCC CTTTAACTTC
      781 CAGGCTGCCT ATGGCCTGAG TGACCAACTG GCCCAAGCCA TCAGTGACCA CTATCCAGTG
      841 GAGGTGATGC TGAAGTGA
```

11

WO 01/74905 PCT/GB01/01324

# 4/113

# pAS6 - light chain

```
18-AUG-1998
            HMFG1LC2.D
                          721 bp
                                     DNA
            HUMANISED HMFG1 LIGHT CHAIN Vnp LEADER.
DEFINITION
ACCESSION
KEYWORDS
SOURCE
  ORGANISM
REFERENCE
            1 (BASES 1 TO 342)
  AUTHORS
            VERHOEYEN ET AL
            CONSTRUCTION OF RESHAPED HMFG1 ETC
  TITLE
            IMMUNOL. (1993):78, 364-370
  JOURNAL
            SCANNED IN FROM JOURNAL
COMMENT
FEATURES
  SITES
```

This is the sequence of the HMFG1 light chain gene with the Vnp leader sequence attached. Translate from residue 1. Note residue 399 is T > A in all clones leading to R133 silent mutation (T in Verhoeyen paper)

BASE COUNT 197 a 202 c 182 g 140 t ORIGIN ?

```
1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCGGAC
61 ATCCAGATGA CCCAGAGCCC AAGCAGCCTG AGCGCCAGCG TGGGTGACAG AGTGACCATC
121 ACCTGTAAGT CCAGTCAGAG CCTTTTATAT AGTAGCAATC AAAAGATCTA CTTGGCCTGG
181 TACCAGCAGA AGCCAGGTAA GGCTCCAAAG CTGCTGATCT ACTGGGCATC CACTAGGGAA
241 TCTGGTGTGC CAAGCAGATT CAGCGGTAGC GGTAGCGGTA CCGACTTCAC CTTCACCATC
301 AGCAGCCTCC AGCCAGAGGA CATCGCCACC TACTACTGCC AGCAATATTA TAGATATCCT
361 CGGACGTTCG GCCAAGGGAC CAAGGTGGAA ATCAAACGAA CTGTGGCTGC ACCATCTGTC
421 TTCATCTTCC CGCCATCTGA TGAGCAGTTG AAATCTGGAA CTGCCTCTGT TGTGTGCCTG
481 CTGAATAACT TCTATCCCAG AGAGGCCAAA GTACAGTGGA AGGTGGATAA CGCCCTCCAA
541 TCGGGTAACT CCCAGGAGAG TGTCACAGAG CAGGACAGCA AGGACAGCA CTACAGCCTC
601 AGCAGCACCC TGACGCTGAG CAAAGCAGAC TACGAGAAAC ACAAAGTCTA CGCCTGCGAA
661 GTCACCCATC AGGGCCTGAG CTCGCCCGTC ACAAAGAGCT TCAACAGGGG AGAGTGTTAG
```

Fig. 3(A)

# 5/113

```
29-AUG-2000
                                                    SYN
           HHMFGlKLC
                         730 BP SS-DNA
LOCUS
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                     Location/Qualifiers
FEATURES
                     10..730
     frag
                     /note="1 to 721 of hHMFGllight chain"
                     10..730
     frag
                     /note="1 to 72 of 104linker"
                     join(10..>63,<65..81)
     frag
                     /note="1 to 72 of 103linker [Split]"
                     join(10..>60,<61..>63,<65..81)
     frag
                     /note="1 to 78 of 102linker [Split]"
                                                      0 OTHER
                                           140 T
                        208 C
                                  184 G
BASE COUNT
                198 A
ORIGIN
        1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCGACA TCCAGATGAC CCAGAGCCCA AGCAGCCTGA GCGCCAGCGT GGGTGACAGA
      121 GTGACCATCA CCTGTAAGTC CAGTCAGAGC CTTTTATATA GTAGCAATCA AAAGATCTAC
      181 TTGGCCTGGT ACCAGCAGAA GCCAGGTAAG GCTCCAAAGC TGCTGATCTA CTGGGCATCC
      241 ACTAGGGAAT CTGGTGTGCC AAGCAGATTC AGCGGTAGCG GTAGCGGTAC CGACTTCACC
      301 TTCACCATCA GCAGCCTCCA GCCAGAGGAC ATCGCCACCT ACTACTGCCA GCAATATTAT
      361 AGATATCCTC GGACGTTCGG CCAAGGGACC AAGGTGGAAA TCAAACGAAC TGTGGCTGCA
      421 CCATCTGTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCCTCTGTT
      481 GTGTGCCTGC TGAATAACTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
      541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
      601 TACAGCCTCA GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
      661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
      721 GAGTGTTAGA
11
```

# Fig. 3(B)

# 6/113

# HMFG-1 light chain with Vnp Leader (shaded)

MGWSCHE VALAFGWESDIQMTQSPSSLSASVGDRVTITCKSSQSL LYSSNQKIYLAWYQQKPGKAPKLLIYWASTRESGVPSRFSGSGSGT DFTFTISSLQPEDIATYYCQQYYRYPRTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEC

Fig. 3(C)

WO 01/74905 PCT/GB01/01324

# 7/113 pAS6 – heavy chain

```
DNA
                                                             14-AUG-1998
            HHMFG1HC.D
                        1404 bp
DEFINITION HUMANISED HMFG1 heavy chain
ACCESSION
           HHMFG1H
KEYWORDS
SOURCE
  ORGANISM
REFERENCE
            VERHOEYEN ET AL
 AUTHORS
            CONSTRUCTION OF RESHAPED HMFG1 etc
 TITLE
            IMMUNOL. (1993):78, 364-370
 JOURNAL
            VH domain SCANNED IN FROM JOURNAL
COMMENT
            AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES
            Residue 963 is G > T leading to silent mutation in all clones
FEATURES
  SITES
BASE COUNT
                333 a
                         439 c
                                  379 q
                                          253 t
ORIGIN
                 ?
                                  LEADER
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACAFGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
      781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
      841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
      901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
      961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
     1021 TGCAAGGTCT CCAACAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
     1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
     1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
     1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
     1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
     1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
     1381 CTCTCCCTGT CTCCGGGTAA ATGA
                                        Antibody DNase Fusions Made Here
                                        (eg pAS34----39.)
         End of lower hinge region of heavy chain. PAPE Amino
         Acid Seq. Fab'2 fusions were made at this point.
         Those with HYBRID HINGES are altered further
         up
        This part
                   GACAAAACTGACACA
```

After this sequence you get the HYBRID HINGE + LINKER SEQUENCES Then DNAse I (eg Fab-DNase construct pAS302)

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GCA CCT GAA GGG AGC GGC GGG CTG AAG ATC GCA GCC TTC AAC CGT GGA CTT CTAG

A P E | G S G G L K I A A F N GAG AGG GAC AGA GGG TAAAN GGG AGG GGG GGG CTG AAG AGG CCA TTT CCC TCG CCG CCC GAC TTC TAG

L S L S P G K G S G G L K I A A F N z 🖡 HAA GGG AGC GGG CTG AAG ATC GCA GCC TTC AAC
TTT CCC TCG CCG CCC GAC TTC TAG

K G S G G L K I A A F N
LINKER hu DNASS I CTG AAG ATC GCA GCC TTC AAC GAC TTC TAG hu DNAse I hn DNAse Oligos involved in the fusion of whole antibody-DNase LINKER LINKER Oligos involved in the fusion of Fab'2-DNaseI 666 AGC 6GC 6G6 CCC TCG CCG CCO 6 S G G AG (deletion) AG (deletion) GAG AGG GAC AGA GGC L L S L S P HMFG1 GAG AGG GAC AGA GGC HMFG1 HINGE GGT GGC ACG GGT HMFC-1 Constructs pAS35/38 Constructs pAS36/39 Constructs pAS34/37 Constructs pAS23/27 ບ а AS79 AS80 AS81 AS82 AS73 AS74 AS83 AS84

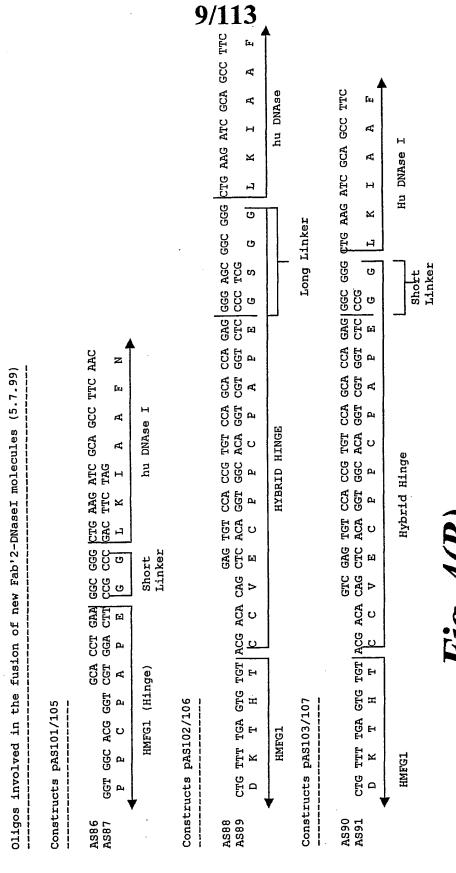


Fig. 4(B)

WO 01/74905 PCT/GB01/01324

# 10/113

## pAS23

```
06-MAR-1995
LOCUS
            PAS23. DNA
                         1554 bp
                                    mRNA
                                                    PRI
           Humanised HMFG1 Fab'2 fused to human DNase I (construct 1)
DEFINITION
ACCESSION
NID
KEYWORDS
            DNase I.
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
MHDNASE1.dna)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic
  TITLE
fibrosis ·
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
 MEDLINE
            91067672
                                           308 t
BASE COUNT
                344 a
                         468 c
                                  434 a
ORIGIN
       1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
     121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
     181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
     241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
     301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
     361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
     541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC
     781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
     841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
     901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
```

Fig. 5(A)

//

961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GTGA

# 11/113

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1554 BP SS-DNA
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                                                              25-AUG-2000
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SOURCE
                    Location/Qualifiers
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                     721..786
     frag
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                     join (721..>735,<736..786)
     frag
                     /note="1 to 78 of 102linker [Split]"
                                 435 G
                                          309 T
                         466 C
BASE COUNT
                344 A
ORIGIN
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       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GGAGCGGCGG GCTGAAGATC
      781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
      841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
      901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
      961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
     1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
     1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
     1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
     1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
     1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
     1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
     1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
     1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
     1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GTGA
11
```

Fig. 5(B)

WO 01/74905 PCT/GB01/01324

# 12/113

```
FDDNASE23K 1563 BP SS-DNA
LOCUS
                                                    SYN
                                                              29-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
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                     /note="1 to 1554 of 23.dna [Split]"
                     730..795
     frag
                     /note="1 to 66 of 23/27linker"
                     join(730..>744,<745..795)
     frag
                     /note="1 to 78 of 102linker [Split]"
               345 A
BASE COUNT
                         472 C
                                 437 G
                                          309 T
                                                      0 OTHER
ORIGIN
       1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
      181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
      301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
      361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
      481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
      541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCCTTGGG CACCCAGACC
      661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
      721 AAATCTTGTG ACAAAACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG GAGCGGCGGG
      781 CTGAAGATCG CAGCCTTCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC
      841 CTCGTCAGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC
     901 AGAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA
     961 GACACCTATC ACTACGTGGT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC
    1021 CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC
    1081 TGCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTCAG GTTCTTCTCC
    1141 CGGTTCACAG AGGTCAGGGA GTTTGCCATT GTTCCCCTGC ATGCGGCCCC GGGGGACGCA
    1201 GTAGCCGAGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAAGAGAA ATGGGGCTTG
   1261 GAGGACGTCA TGTTGATGGG CGACTTCAAT GCGGGCTGCA GCTATGTGAG ACCCTCCCAG
    1321 TGGTCATCCA TCCGCCTGTG GACAAGCCCC ACCTTCCAGT GGCTGATCCC CGACAGCGCT
    1381 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
    1441 CTCCGAGGGG CCGTTGTTCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC
    1501 CTGAGTGACC AACTGGCCCA AGCCATCAGT GACCACTATC CAGTGGAGGT GATGCTGAAG
    1561 TGA
11
```

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					j	3/	LI	3									
		9			18			27		٠	36						54
ATG	GGA	TGG .	AGC	TGT	ATC	ATC			TTG	GTA	GCA	ACA	GCT	AÇA	GGT	GTC	CAC
 M	 G	 W	s	C	I	 I	L	F	L	· v	A	T	A	T	G	v	. н
					72			81			90			99	•		108
ጥርር	CAG	63 GTG	CAG	CTG		CAG	TCT		GCA	GAG		AAA	AAG	CCT	GGG	GCC	TCA
				- <u>"</u> -													
.s	Q	v	Q	L	v	Q	S	G	A	E	V	K	K	P	G	A	S
		117			126			135						153			162
GTG	AAG	GTG	TCC	TGC	-					ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAC
. v			 s	 С	 к	 _A		G		T	F	s	A	Y	W	I	E
		171			180			189			198			207			216
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CC
 W	 v	 R	<del></del> -	 A	 P	 G	 K	 G	.F	 E		v	 G	E	I.	L	P
		225			234			243			252			261			27
GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	AAG	TTC			CGA	GTG	ACA	GTC	ÀC:
<b>-</b> G	 S	 N	 N	 s	 R	 У	n	 E	к	F	к		R	v	т	v	T
•		279			288			297			306			315			32
AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC			GAG			AGC	CTG	AGG	TCT	GA
 R		 T	 s	 T	 N	 T	- <u>-</u> -	 Y	 M	E	L	 S	s	L	R	 s	E
		•	•		242			251			360			369	•		37
CAC	מיימ	333	כיזיכ	тат	342 TAC								GCC			GCT	
.D	T	A	v	Y.	Y	С	A	R	S	Y	D	F	A	W	F	A	Y
		387	,		396			405			414			423			43
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TC
W	 G	Q	G	· T	L	v	т	v	S	S	A	s	T	К	G	. <b>P</b>	s
	•	44]	ı		450			459			468	}		477	,		48
GTC	TTC	CCC	CTG	GCA	222	TCC	TCC			ACC	TCI	GGG	GGC	: ACA	GCG	GCC	CI
	·												·			 A	 I
¥	F	P	L	A	P	S	S	K	5	T		G	G	1	^	n	
		49	5		504			513			522		- CW	531			54 יידרי
GGC	TG	C. CT	G GT	AAC	GAC	TAC	TTC	: CCC	; GA2		. GIC	ACC	, 616	, 100		AAC	. IX
G	С	L	 V	ĸ	ם	Y	F	P	E	P	V	T	v	s	W	N	5
		54	9		558			567	7		570	5.		585	5		59
GG	C GC	ידכ ידס מ	G AC	C AGO			CAC			c cc			CT?	A CAC	TCC	TCF	4 GC
		L		s	_			_	_	P				_	S	S	

Fig. 5(D) (Sheet 1 of 3)

								Т.	Ŧ/ J		,							
			603			612			621						639			648
	TC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GIG	ccc	TCC	AGC	AGC	TTG	GGC	ACC	CAG
•	r.	Y	s	L	s	s	v	v	T	v	P	s	s	s	L	G	T	Q
	-	-	Ū	-	-		•	·	•	•	•	J	J	J		٥	•	Q
			657			666			675			684			693			702
F	CC	TAC	ATC	TGC	AAC	GTG	TAA	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	A.A.A
-																		
	Т	Y	I	С	N	ν	N	Н	K	P	S	N	T	K	V.	D	K	K
			711		•	720			729			738			747			756
C	тт	GAG		AAA	TCT	TGT		AAA			ACA			CCG			GCA	
-																		
	V	E	P	K.	s	С	D	K	T	H	T	С	P	P	С	P	A	₽
																	•	
,	ת תי	000	765	~~~		774		3000	783		mmc	792			801			810
-			AGC			CTG	AAG	A1C	GCA			AAC	AIC	CAG	ACA	111	GGG	GAG
	E	G	s	G	G	L	K	I	A	A	F	и	I	Q	T	F	G	E
														7	_	_	_	-
			819			828			837			846			855			864
7	rCC	AAG	ATG	TCC	TAA	GCC	ACC	CTC	GTC	AGC	· TAC	TTA	GTG	CAG	ATC	CTG	AGC	CGC
-	т		- M	 s	N	Α	т	L	v	s		ı	v					
,	•			3	74		•	L	٧	3	1	_	v	Q	I	L	S	R
			873			882			891			900			909			918
1	'AC	GAC	ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG
-																		
	1	ע	7	A	ь	V	Q	E	V	К	D	S	н	L	. <b>T</b>	А	V	G
			927			936			945			954			963			972
A	AG	CTG	CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GTG	
-									·									
٠	K	L	L,	D	N	L	N	Q	D	A	P	. D	T	Y	H	Y	V	V
		•	981	•		990			999		1	1008		,	1017		,	1026
A	GT.	GAG		CTG	GGA	CGG	AAC	AGC		AAG			TAC			GTG		
-																		
	S	E	₽	L	G	R	N	s <sub>.</sub>	Y	K '	£	R	Y	L	F	V	Y	R
			L035			1044		. ,	1053		,	.062		,	.071			
С	CT			GTG		GCG	GTG			TAC			GAT			TGC		080
	P	D	Q	ν	S	A	v	D	s	Y	<b>Y</b> ·	Y	D	D	G	С	E.	P
			000					_						_			_	
т	CC.		089 244			1098 TTC												134
_																		
	Ç	G	· N	D	T	F	N	R	£	P	A	ı	v	R	F	F	s	R
•														•				
•	<b>~</b> ~		1143	OTP.O		1152			161			170	a		179	000		188
-	1C					GAG					ccc	CTG	CAT	GCG	GCC	CCG	GGG	GAC
	F					Ë						L	н	A	A	P	G	D
•																		-
		1	197			206	_					224		1	233		1	242
						F	io	_	5	<b>(1</b>	"							
					-	F	5	•	J (		"							•
					4	SI	10	2 <b>f</b>	2 /	f	<b>2</b> 1							
					•	IJ	ょせも	~ I	4 C	η.	JI							

GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA A V A E I D A L Y D V Y L D V · Q E K 1260 1269 1278 1287 1296 TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT --- --- --- --- --- --- --- --- --- --- --- --- --- ---W G L E D V M L M G D F N. A G C S Y 1314 . 1323 1332 1341 GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 1359 1368 1377 1386 1395 TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT W L I P D S A D T T A T P T H C A Y. 1413 1422 1431 1440 1449 1458 CAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT GTT CCC GAC TCG D R I V V A G M L L R G A V V P D S 1485 1494 1503 1512 1476 GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC CAA CTG GCC CAA \_\_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ 1530 1539 1548 GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG TGA 3' A I S D H Y P V E V M L K \*

Fig. 5(D) (Sheet 3 of 3)

### 16/113

#### pAS27

```
PAS27.DNA
                        1584 bp
                                   mRNA
                                                    PRI
                                                              06-MAR-1995
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I with SV40
NLS(construct 1)
ACCESSION
NID
KEYWORDS
            DNase I.
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
MHDNASE1.dna)
  ORGANISM Homo sapiens
           Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
           Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
 AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic fibrosis
  TITLE
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
            91067672
  MEDLINE
                        474 c
                                  446 g
                                           310 t
                354 a
BASE COUNT
ORIGIN
       1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
     421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
     481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
     541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GGAGCGGCGG GCTGAAGATC
      781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
      841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
      901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
      961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
    1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
    1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
    1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
    1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
    1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
    1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
    1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
    1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
    1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGGCGGA
    1561 CCCAAAAGA AGCGCAAGGT TTGA
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### 17/113

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25-AUG-2000
                       1584 BP SS-DNA
                                                   SYN
           FDDNASE27
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
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                     /note="1 to 1584 of 27.dna [Split]"
                     721..786
    frag
                     /note="1 to 66 of 23/27linker"
                     join(721..>735,<736..786)
    frag
                    /note="1 to 78 of 102linker [Split]"
                                           311 T
                                  447 G
                        472 C
BASE COUNT
ORIGIN
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       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GGAGCGGCGG GCTGAAGATC
      781 GCAGCCTTCA ACATCCAGAC ATTTGGGGAG ACCAAGATGT CCAATGCCAC CCTCGTCAGC
      841 TACATTGTGC AGATCCTGAG CCGCTACGAC ATCGCCCTGG TCCAGGAGGT CAGAGACAGC
      901 CACCTGACTG CCGTGGGGAA GCTGCTGGAC AACCTCAATC AGGACGCACC AGACACCTAT
      961 CACTACGTGG TCAGTGAGCC ACTGGGACGG AACAGCTATA AGGAGCGCTA CCTGTTCGTG
     1021 TACAGGCCTG ACCAGGTGTC TGCGGTGGAC AGCTACTACT ACGATGATGG CTGCGAGCCC
     1081 TGCGGGAACG ACACCTTCAA CCGAGAGCCA GCCATTGTCA GGTTCTTCTC CCGGTTCACA
     1141 GAGGTCAGGG AGTTTGCCAT TGTTCCCCTG CATGCGGCCC CGGGGGACGC AGTAGCCGAG
     1201 ATCGACGCTC TCTATGACGT CTACCTGGAT GTCCAAGAGA AATGGGGCTT GGAGGACGTC
     1261 ATGTTGATGG GCGACTTCAA TGCGGGCTGC AGCTATGTGA GACCCTCCCA GTGGTCATCC
     1321 ATCCGCCTGT GGACAAGCCC CACCTTCCAG TGGCTGATCC CCGACAGCGC TGACACCACA
     1381 GCTACACCCA CGCACTGTGC CTATGACAGG ATCGTGGTTG CAGGGATGCT GCTCCGAGGG
     1441 GCCGTTGTTC CCGACTCGGC TCTTCCCTTT AACTTCCAGG CTGCCTATGG CCTGAGTGAC
     1501 CAACTGGCCC AAGCCATCAG TGACCACTAT CCAGTGGAGG TGATGCTGAA GGGGGGCGGA
     1561 CCCAAAAAGA AGCGCAAGGT TTGA
```

### 18/113

```
1593 BP SS-DNA
                                                    SYN
                                                              29-AUG-2000
           FDDNASE27K
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DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
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                    join(10..>729,<796..1593)
     frag
                     /note="1 to 1584 of 27.dna [Split]"
                    730..795
     frag
                     /note="1 to 66 of 23/27linker"
                     join(730..>744,<745..795)
     frag
                     /note="1 to 78 of 102linker [Split]"
BASE COUNT
                        478 C
                                  449 G
                                           311 T
ORIGIN
       1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
      61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
     121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
     421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
     541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
     601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
     661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
     721 AAATCTTGTG ACAAAACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG GAGCGGCGGG
     781 CTGAAGATCG CAGCCTTCAA CATCCAGACA TTTGGGGAGA CCAAGATGTC CAATGCCACC
     841 CTCGTCAGCT ACATTGTGCA GATCCTGAGC CGCTACGACA TCGCCCTGGT CCAGGAGGTC
     901 AGAGACAGCC ACCTGACTGC CGTGGGGAAG CTGCTGGACA ACCTCAATCA GGACGCACCA
     961 GACACCTATC ACTACGTGGT CAGTGAGCCA CTGGGACGGA ACAGCTATAA GGAGCGCTAC
     1021 CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT GCGGTGGACA GCTACTACTA CGATGATGGC
     1081 TGCGAGCCCT GCGGGAACGA CACCTTCAAC CGAGAGCCAG CCATTGTCAG GTTCTTCTCC
     1141 CGGTTCACAG AGGTCAGGGA GTTTGCCATT GTTCCCCTGC ATGCGGCCCC GGGGGACGCA
     1201 GTAGCCGAGA TCGACGCTCT CTATGACGTC TACCTGGATG TCCAAGAGAA ATGGGGCTTG
    1261 GAGGACGTCA TGTTGATGGG CGACTTCAAT GCGGGCTGCA GCTATGTGAG ACCCTCCCAG
    1321 TGGTCATCCA TCCGCCTGTG GACAAGCCCC ACCTTCCAGT GGCTGATCCC CGACAGCGCT
    1381 GACACCACAG CTACACCCAC GCACTGTGCC TATGACAGGA TCGTGGTTGC AGGGATGCTG
    1441 CTCCGAGGGG CCGTTGTTCC CGACTCGGCT CTTCCCTTTA ACTTCCAGGC TGCCTATGGC
    1501 CTGAGTGACC AACTGGCCCA AGCCATCAGT GACCACTATC CAGTGGAGGT GATGCTGAAG
    1561 GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT TGA
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						-	LY		IJ								
		9			18			27			36			45			54
ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
M	G .	W	s	С	I	I.	L	F	L	V	Α	T	A	T	G	V	н
			٠.								90			99			108
	~~~	63	CNC	C4IV	72	CAC	mΩm.	81	CCA	GAG		444	DA4		GGG	GCC	
TCC	CAG	616	CAG														
s	Q	v	Q	L	v	Q	s	G	A	E	v	ĸ	K	P	G	A	s
		117			126			135			144			153	maa		162
GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TIC	AGT	GCC	TAC	TGG	ATA	GAG
v	ĸ	v	s	С	ĸ	A	s	G	Y	T	F	s	A	Y	W	I	E
													•				
		171			180			189			198			207			216
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
w	v	R	Q	A	P	G	ĸ	G	L	E	W	v	G	E	I	L	P
								040			252			261			270
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GGA	AGT	AAT	AAT	101	AGA												
G	s	N	N	s	R	Y	. N	E	ĸ	F	ĸ	G	R	v	T	v	T
					200			297			306			315			324
202	CAC	279		202	288	ארא	GCC			GAG		AGC	AGC		AGG	TCT	
														·			
R	D	T	s	T	N	T	A	Y	M	E	L	S	s	L	R	s	E
		333			342			351			360			369			378
GAC	ACA			TAT			GCA			TAC			GĊC	TGG	TTT	GCT	TAC
																	-,
D	T	A	v	Y	Y	С	A	R	S	Y	D	F	A.	W	F	A	Y
		387			396			405			414			423			432
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
																P .	
W	G	Q	G	T	L	V	T	V	S	s	A	S	T	K	G	P	S
	•	441			450			459			468			477			486
GTC	TTC			GCA	ccc	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ДСА	GCG	GCC	CTG
			,-														
V	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
		495												531			540
GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	ccc	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA
G	С	L	V	K	D	Y	F	P	£	₽	V	Т	V	S	₩	N	S
		549			558			567			576			585			594
															TCC	TCA	GGA
					_			_	_	_	_	v	-	_	_	_	

Fig. 6(D) (Sheet 1 of 3)

		603			612			621			630			639			648
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TIG	GGC	ACC.	CAG
	Y	s	L	s	s	٠V	v	T	v	P	s	s	s	L	G	T	Q
											<b>504</b>			603			700
»CC	ጥልሮ	657	TCC	244	666 CTC		CAC	675 AAG	CCC	AGC			AAG		GAC	AAG	•
T	Y	I	С	N	v	N	Н	K	₽	S	N	T	К	. <b>v</b>	D	K	K
		711			720			729			738			747			756
GTT	GAG		AAA	TCT		GAC	AAA		ĊAC	ACA		CCA	CCG		CCA	GCA	
V	E	P	K	S	С	D	K	T	н	T	С	P	₽	С	P	A	P
		765			774			783			792			801			810
GAA	GGG	AGC	GGC	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG
 E	 G	 s			т.		т						0	T	 F	G	
L	G	3	G	J		•	-	••	••	-	-		•	-	_	•	_
		819			828			837			846			855			864
ACC	AAG	ATG	TCC	TAA	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	CTG	AGC	CGC
T	K	м	S	N	A	T	L	v	s	Y	I	v	Q	I	L	S	R
ጥልሮ	GAC	873		CTC	882 CTC			891 GTC		GAC	900 AGC		CTG	909 ACT	GCC	GTG	918 GGG
Y	D	I	A	L	V	Q	E	V	R	D	s	Н	L	T	A	V	G
		927			936			945			954			963			972
AAG	CTG			AAC			CAG		GCA	CCA		ACC	TAT		TAC	GTG	-
K	L	L	D	N	. L	N	Q	D	A	Р	ט	T	Y	Н	Y	V	V
		981			990			999			1008			1017			1026
AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG	TAC	AGG
s		. P	L	G	R	N	s	Y	К	E	R.	Y	L	F	v	Y	R
										•							
000		1035			1044			1053 ACC							TGC		1080
	GAC	CAG		101													
P	D	Q	v	s	A	v	D	s.	Y	Y.	Y	D	D	G	С	E	P
		1089			1098			1107			1116	•		1125			1134
TGC															TŤC		
С		N	D	T	F	N	R	E	₽	Α.	1	V	R	r	r	s	ĸ
		1143						1161									1188
TTC	ĄCA	GAG	GTC	AGG	GAG	TTT	GCC	TTA	CTT	ccc	CTG	CAT	GCG	GCC	CCG	GGG	GAC
 F	ጥ	E	 V	 R		 F	 A	 I	 v	 P	L	н	 A	Α	P	 G	D
•	•	_															
								1215			1224			1233			1242
		I	$\vec{i}$	Œ	•	<b>\</b> /	D	)		•	2 -						
		L	$\cdot \boldsymbol{\nu}$	<b>~</b> •	U	"	J	•									

Fig. 6(D) (Sheet 2 of 3)

GCA	GTA	GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA	GAG	AAA
A	v	Α	E	I	D	A	L	Y	D	v	Y	L	Đ	v	Q	Ε	К
		1251		1	1260		1	1269		1	1.278		:	1287			1296
TGG							TTG	ATG	GGC	GAC	TTC	TAA	GCG	GGC	TGC		
w	 G	L	E	ם	v	 М								G		s	Y
	:	1305			1314		:	1323			1332		- :	1341		:	1350
GTG	AGA	ccc	TCC	CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	ccc	ACC	TTC	
v	 R	P												P			Q
	:	1359		:	1368		:	1377			1386		:	1395		:	1404
TGG						GCT	GAC							CAC			TAT
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		1413	•		1422			1431		:	1440		:	1449		:	1458
GAC														GTT			
D	 R		v		 A	 G								v			
		1467		,	1476			1485			1494			1503			1512
GCT														CAA	CTG	GCC	CAA
 A	L	 P	F	N	 F	Q	 A	A .	 Y	 G		s	D	Q		A	Q
		1521			1530	•		1570			1548			1557			1566
GCC														GGC			
A	I	S	D	H	Y	P	. <b>v</b>	E	V	M	Ŀ	К		G		P	
		1575			1584												
AAG			AAG			3,											
K			к							٠							

Fig. 6D (Sheet 3 of 3)

### 22/113 pAS34

LOCUS PAS34.DNA 2196 bp 2196 bp 2196 bp DNA 14-AUG-1998 HUMANISED HMFG1 heavy chain fused to human DNAse construct 34 DEFINITION DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file) REFERENCE VERHOEYEN ET AL AUTHORS CONSTRUCTION OF RESHAPED HMFG1 etc TITLE JOURNAL IMMUNOL. (1993):78, 364-370 COMMENT Human DNAse sequence is modified as a result of oligo assembly (mhdnase.dna) COMMENT The fusion was made using overlapping oligos AS79 and AS80 AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) FEATURES **FEATURES** Residue 963 is G > T leading to silent mutation in all clones SITES Note BASE COUNT 501 a 677 c 607 g 411 t

ORIGIN

11

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGGGTAA A<u>GGGAGCGGC GGG</u>CTGAAGA TCGCAGCCTT CAACATCCAG 1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG 1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG 1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG 1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG 1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC 1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC 1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC 1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC 1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC 1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT 2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG 2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC 2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGTGA

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		9			18		~~~	27	<b>4</b> 000	OM 3	36	202		45	CCT	GTC	5
ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	77G	GTA	GCA					GTC	
H	G	W	s	С	I	I	L	F	L	V	A	T	A	T	G	V	<u> </u>
		63			72			81			90			99			10
TCC	CAG	GTG 	CAG	CTG	GTG	CAG	TCT	GGG 	GCA	GAG	GTG	AAA	AAG			GCC	
<u>, s</u>	Q	v	Q	L	v	Q	s	G	A	E	v	K	K	P	G	A	s
		117			126			135			144			153			16
GTG	AAG	GTG	TCC	TGC	AAG 	GCT		GGC			TTC		GCC	TAC	TGG	ATA	GA
v.	ĸ	v	s	С	ĸ	A	s	G	Y	T	F	s	A	Y	W	I	. E
		171			180			189			198			207			21
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CC
W	v	R	Q	A.	P	G	ĸ	G	L	E	W	v	G	E	I	L	F
		225			234			243			252			261			27
GĢA	agt	AAT	AAT	TCT			AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	AC
G	s	Й	N	s	R	Y	N	E	ĸ	F	.K	G	R	v	T	v	7
		279			288			297			306			315			32
AGA	GAC	ACA	TCC	ACA		ACA	GCC		.ATG	GĄG	CTC	AGC	AGC	CTG	AGG	TCT	G?
R	D	T	5	T	N	т.	A	Y	M	E	L	s	ŗs	r	R	s	E
		333			342			351			360			369			37
			GTC	TAT		TGT	GCA			TAC			GCC	TGG	TTT	GCT	T
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		387			396			405			414			423			43
TGG	GGC		GGG	ACT			ACA		TCC	TCA			.ACC		GGC	CCA	TC
w	G	Q	G	T	L	v	T	v	s	s	Α	s	T	к	G	P	
•		441			450			459			468			477			48
GTC	ŢŢC		CTG	GCA			TCC	AAG	AGC	ACC			GGC			GCC	
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		495			504			513			522			531			54
GGC	TGC				GAC	TAC		CCC	GAA		GTG	ACG		TCG	TGG	AAC	
·		L	v										v			N	
		549			558			567			576			585			59
GGC	GCC			AGC			CAC			ccc			CTA			TCA	
 G		L	T	s	G	v	н	T	F	P	A	v	ŗ.	. Q	s	s	
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CTC	TAC	TCC	CTC	AGC			GTG				TCC	AGC	AGC			ACC	
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ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSN1KVDKK 729 711 720 738 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT V E P K S C D K T H T C P P C P A P 774 783 792 765 801 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT 828 837 846 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GAC GTG AGC CAC LMISRTPEVTCVVVDVSH 873 882 891 900 909 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT EDPEVKFNWYVDGVEVHN 945 927 936 954 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC AKTKPREEQYNSTYRVVS . 999 990 1008 1017 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H O D W L N G K E Y K C K 1035 1044 1080 1053 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T I S K A K 1098 1107 1116 1089 1125 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG GQPREPQVYTLPPSRDEL 1143 .1152 1161 1170 .1179 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---T K N Q V S L T C L V K G F Y P S D 1197 · 1206 1215 1224 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG I A V E W E S N G Q P E N N Y K T T 1260 1269 1251 1278 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDG·SFFLYSKLTV 1323 1305 1314 . 1332 1341 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG D K S R W Q Q G N V F S C S V M H E

Fig. 7(B)
(Sheet 2 of 4)

- 2 -

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	3	1359		1	368		1	377		1	1386		1	1395		3	1404
GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCG	GGT	AAA	GGG
A	L	н	N	н	Y	T	Q	К	s	L	s	L	s	P	G	K	G
	1	1413		1	422		1	431		1	1440		3	L449		1	1458
AGC	eec.	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG	ACC	AAG
c	G	G	L	К	I	A	A	F	N	I	Q	T	F	G	E	T	K
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		1467		1	476		1	485		1	1494		1	1503	•	7	1512
A TYC	TCC.	ስ እጥ	GCC			CTC	ACC.	TAC	ATT	GTG	CAG	ATC	CTG	AGC	CGC	TAC	GAC
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		1521		,	L530		1	L539		-	1548		1	1557			1566
	~~~	-m-	cmc			CTC.			»cc						GGG		
ATC	GCC	CIG	GIC	CAG	GAG	GIC	AGA	GAC									
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I	A	L	V	Q	E	v	R	ט	3	n		•	-	•	·	•••	-
								1593			1602			1611		٠.	1620
		1575			1584				C) C			CAC			GTC		
CTG	GAC	AAC	CIC	AAT.	CAG	GAL	GLA	CCA	GAC	ACC							
								P	D	т	Y	н	Y	v	٧	s	Е
L	D	N	L	N	Q	D	A	2	ע	1	•	п	-	٠	٠	-	
											1656			1665			1674
		1629			1638			1647				mmc			NCC		
CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	الحال	TAC	CIG	110	GIG	THE	AGG	CCI	GAC
											L	F	v	Y	R	P	D
P	L	G	R	N	s	Y	ĸ	B	R	Y	ъ	r	•	7	А	P	υ
											1710			1719			1728
		1683			1692			1701			1710						
CAG	GTG	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT	كاباقا	160	GAG	ccc	TGC	666
																	G
Q	v	S	A	V	D	s	Y	Y	Y	D	D	G	С	E	P	С	G
											1764			1773			1782
		1737			1746		:					mmc			~~		
AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT	GIC	AGG	TIC	110	100	CGG	IIC	ACA
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N	D	T	F	N	R	E	P	A	1	v	R	r	F	3	K	F	
•														1827		:	1836
		1791			1800			1809			1818	000			CNC		
GAG	GTC	AGG	GAG	TTT	GCC	ATT	GIT	CCC	CIG	CAT	GCG	GCC	CCG	666	GAC	GCA	
												A	-;- P	G	D	A	ν
E	V	R	E	F	A	I	v	P	L	Н	A	A	E	•	٠	^	•
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		1845			1854	m » m			ጥአሮ						AAA		
GCC	GAG	ATC	GAC	GCT	CIC	TWL	GAC	G1C							~		
			D	Α	L	Y	D	v	Y	L	D	v	Q	E	K	W	G
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		1899			1908			1917			1926			1935			1944
marks.	CAC	1022	CTC	ስጥር	באת		GGC	GAC	TTC	TAA					TAT	GTG	AGA
116	GAG																
	F	ת	v	м	t.	м	G	D	F	N	A	G	С	s	Y	v	R
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		1953			1962			1971			1980			1989			1998
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P												P	T	F	Q	W	L
•	_	¥	••	-	-	_		_	-	-					-		
								2025			2034			2043			2052
		2007			2016												
ንሞል	ecc	2007 GAC	വാക	GCT	GAC	ACC	ACA	GCT	AÇA	CCC	ACG	CAC	TGT	GCC			AGG
ATC	: ccc	2007 GAC	AGC	GCT	GAC	ACC	ACA	GCT	ACA	ccc	ACG	CAC	TGT	GCC	TAT	GAC	AGG
		GAC	AGC	GCT	GAC	ACC	ACA				·				TAT	GAC	
		GAC	AGC S	GCT	GAC D	ACC T	ACA T	Α	T	P	·				TAT	GAC	

Fig. 7(B) (Sheet 3 of 4)

	:	2061		-	2070		:	2079		:	2088		2	2097		- 1	2106
ATC	GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	GAC	TCG	GCT	CTT
I	v	V	Α	G	M	L	L	R	G	A	V	V	P	D	S	A	L
	:	2115	5 2124 C TTC CAG GCT G				:	2133		- :	2142		- 2	2151		- 3	2160
CCC	TTT	AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC
b.	F	N	F	Q	A	A	Y	G	L	s	D	Q	L	A	Q	A	ı.
	:	2169		- 1	2178	•	2	2187		7	2196						
AGT	GAC	CAC	TAT	CCA	GTG	GAG	GTG	ATG	CTG	AAG	TGA	3'					
s	D	H	Y	P	v	E	v	M	$\mathbf{L}$	K	*						

Fig. 7(B) (Sheet 4 of 4)

### 27/113 pAS35

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2193 bp
                                              DNA
                                                              14-AUG-1998
                        2193 bp
           PAS35.DNA
LOCUS
           HUMANISED HMFG1 heavy chain fused to human DNAse construct 35
DEFINITION
           Clone 17.12.1 with silent K to K mutation (1398 A > G)
DEFINITION
REFERENCE
           VERHOEYEN ET AL
 AUTHORS
           CONSTRUCTION OF RESHAPED HMFG1 etc
  TITLE
           IMMUNOL. (1993):78, 364-370
  JOURNAL
           Human DNAse sequence is modified as a result of oligo assembly
COMMENT
(mhdnase.dna)
           The fusion was made using overlapping oligos AS81 and AS82
COMMENT
           AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES
           Residue 963 is G > T leading to silent mutation in all clones
FEATURES
            In 17.12.1 residue 1398 is A > G (silent K to K mutation)
FEATURES
  SITES
           Note
                                  606 a
                                           410 t
                         677 c
BASE COUNT
                500 a
                 ?
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
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61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
781 TTCCTCTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
1381 CTCTCCCTGT CTCCGAAgGG GAGCGGCGGG CTGAAGATCG CAGCCTTCAA CATCCAGACA
1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC
1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG
1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA
1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT
1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC
1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT
1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC
1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT
1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC
1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC
2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT
2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT
2161 GACCACTATC CAGTGGAGGT GATGCTGAAG TGA
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, ,	ATG	GGA	TGC		TG	11 OTA 1		CTC	27 TTC		GT.	3 A GC	6 A AC	A GC	4 C AC.	S A GG1	T GT	54 C CAC
	м	G	W	s	С	I	1	L	F	L	Ų	Α	т	A	т	G	 v	 н
	TCC	CAG	63 GTC		CTO	72 GTG		TC1	8j		GA(	9( G' <b>GT</b> Y		AAA	9: CC:		G GC	108 C TCA
	. <u>s</u>	Q	v	Q	L	v	Q	 S	 G	 A	 E	 V	 к	 К	 P			 S
			117			126			135			144						
	GTG	AAG			TGC			TCI			: ACC	TTC	AGT	GCC	153 TAC	TGC	ATA	162 GAG
	v	к	v	s	С	ĸ	A	s	G	Y	т	F	s	A	Y	W	Ţ	E
	TCC	CTC	171		CC4	180			189			198			207	7		216
											GAG	160	GIC	GGA	GAG	AT1	TT/	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	v	G	E	I	. T	P
	GC3	ልርጥ	225		m~m	234			243			252			261			270
														CGA		ACA	GTC	ACT
	G	S	N	N	S	R	Y	N	E	K	F	K	G	R	v	T	v	Ť
	AGA	CAC	279		ארא	288	ארא	ccc	297	» mc	C10	306	AGC		315			324
													AGC	AGC		AGG	TCT	GAG
	R	D	T	s ·	T	N	T	A	Y	M	E	. L	S	S	L	R	s	E
	GAC	ACA	333 GCC	GTC	ጥልጥ	342 TAC	ጥርጥ	GC A	351	TCC	ጥስር	360	TTT		369			378
															166		GCT	TAC
	D	T	A	V	Y	Y	С	A	R	S	Y	D	F	A	W	F	A	Y
	TGG	GGC	387 CAA	ccc	<b>ው</b> ጥ	396 CTG	ርሞር	202	405 GTC	arc c	TVC A	414	TCC		423			432
										-:				ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	v	T	V	s	s	A	s	T	K	G	P	S
	GTC	ጥጥር	441	CTG	475	450	TCC	TCC	459	200	100	468	GGG		477			486
															ACA	GCG	GCC	CTG
	v	F	P	L	A	P	s	s.	K	s	T	s	G	G	T_	Α.	A	L
	GGC	TGC	495 CTG	GTC	DAA	504 GAC	ገፈጥ	ሙሆር	513	CDD	CCC	522	ACG	-m-	531	maa		540
	G	, ,	L	V									T					•
	GGC		549 CTG	ACC	AGC	558 GGC	GTG	CAC	567 ACC	ጥጥር	CCC	576 GCT	GTC	ርጥ አ	282	TYCC	<b>~</b> ~ »	594
	G	Α.	ւ	Т					T	F	P	A	v	L	Q	s	s	G
	CTC		603 TCC	CTC	AGC	612 AGC	GTG	GTG	621 ACC	GTG	CCC	630 TCC	AGC	OĐA	639 TTG	GGC	204	648 CAG
													 Ś					
										v	P	S	5	S	L	G	T	Q
			657 <b>T</b>			666	_					684			693			702
					<u> </u>		• • • • • • • • • • • • • • • • • • •		, l									

Fig. 8(B) (Sheet 1 of 4)

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHK PSNTK V DKK 747 711 720 729 738 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT 774 783 792 801 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC --- --- --- --- --- --- --- --- --- --- --- --- --- ---ELLGGPSVFLFPPKPKDT 855 837 846 828 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC L M I S R T P E V T C V V V D V S H 882 891 900 873 909 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT EDPEVKFNWYVDGVEVHN 936 945 954 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC --- --- --- --- --- --- --- --- ---` 999 1008 990 1017 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N G K E Y K C K 1071 1053 1062 1035 1044 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T I S K A K 1116 1125 1098 1107 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG G Q P R E P Q V Y T L P P S R D E L 1152 1161 1170 1179 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ \_\_\_ T K N Q V S L T C L V K G F Y P S D 1206 1215 1224 1233 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG I A V E W E S N G Q P E N N Y K T T 1296 1260 1269 1278 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG P P V L · D S D G S F F L Y S K L T V 1314 1332 1341 1305 1323 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG D K S R W Q Q G N V F S C S V M H E

Fig. 8(B) (Sheet 2 of 4)

GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---ALHNHYTQKSLSLSPK<u>C</u>S GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TIT GGG GAG ACC AAG ATG <u>G</u> L K I A A F N I Q T F G E T K M TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC S N A T L V S Y I V Q I L S R Y D I GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG A L V Q E V R D S H L T A V G K L L GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---DNLNQDAPDTYHYVVSEP 1629 ' 1638 1:656 CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG LGRNSYKERYLFVYRPDQ GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC V S A V D S Y Y Y D D G C E P C G N GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG D T F N R E P A I V R F F S R F T E CTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC V R E F A I V P L H A A P G D A V A GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---EIDALYDVYLDVQEKWGL GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC E D V M L M G D F N A G C S Y V 1989 . 1998 TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC · S Q W S S I R L W T S P T F Q W L I CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC PDSADTTATPTHCAYDRI

Fig. 8(B) (Sheet 3 of 4)

	:	2061			2070			2079		_	8802		_	2097		_	2106
GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	ccc	GAC	TCG	GCT	CTT	CCC
٧.	v	A	G	M	L	L	R	G	A	٧	V	P	D	S	A	L	P
												٠.					
		2115		:	2124		:	2133		:	2142		:	2151		:	2160
بالملحلة	244	TTC	CAG	CCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC	AGT
F	N.	F	Q	A	A	¥	G	L	s	D	Q	L	A	Q	A	I	s
		2169			2178		-	2187									
GAC	CAC	TAT	CCA	GTG	GAG	GTG	ATG	CTG	AAG	TGA	3′						
D	н	Y	P	v	E	v	M	L	K	*							

Fig. 8(B) (Sheet 4 of 4)

### 32/113 pAS36

LOCUS 2190 bp 2190 bp PAS36.DNA DNA 14-AUG-1998 DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse - construct 36 DEFINITION Clone 18.24.1 with residue 1392 T > C REFERENCE **AUTHORS** VERHOEYEN ET AL TITLE CONSTRUCTION OF RESHAPED HMFG1 etc IMMUNOL. (1993):78, 364-370 JOURNAL COMMENT Human DNAse sequence is modified as a result of oligo assembly (mhdnase.dna) The fusion was made using overlapping oligos AS83 and AS84 COMMENT AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A) **FEATURES FEATURES** Residue 963 is G > T leading to silent mutation in all clones FEATURES Residue 1392 T > C silent S to S mutation SITES Note BASE COUNT 498 a 678 c 605 g 409 t ORIGIN

1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CcCCG<u>GGGAG CGGCGGG</u>CTG AAGATCGCAG CCTTCAACAT CCAGACATTT 1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC 1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG 1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG 1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG 1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA 1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT 1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC 1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG 1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC 1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT 2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT 2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC 2161 CACTATCCAG TGGAGGTGAT GCTGAAGTGA

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																	:
M	G	W	<u>s</u>	<u> </u>	_ <del>_</del> _	<u> </u>	<u>. L</u>	F	L		A	T	A	T	G	v	-
		63			72			81			90			99			
TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG	GTG	AAA	AAG	CCT	GGG	GCC	
s	Q	V	Q	L	v	Q	s	Ģ	A	E	v	ĸ	ĸ	P	G	A	
		117			126			135			144			153			
GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	
v	ĸ	v	s	С	ĸ	A	s	G	Y	T	F	s	A	Y	W	I	
		171			180		•	189			198			207			
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	
w	v	R	Q		P		ж	G	L L	 E	w	v	G				
		225			234			243		•	252			261			
GGA	AGT		AAT	TCT		TAC	AAT			TTC			CGA			GTC	
G	s	И	N	S	R	Y	N	E	ĸ	F	K	G	R	v	T	V	
	222	279	<b>m</b> 00		288			297		C) 0	306			315			
AGA	GAC	ACA	100	ACA	AAC	ACA		TAC	ATG	GAG			AGC	CTG	AGG	TCT	
R	D	T.	Ė	Ŧ	N	T	A	Y	M	Е	L	s	s	L	R	s.	
		333			342			351			360			369			
GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	•
D	T	A	v	Y	¥	·C	A	R	s	Y	D	F	A	W	F	A	
		387			396			405			414			423			
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	
W	G	Q	G	T	L	v	T	v	s	s	A	s	T	ĸ	G	P	
		441			450			459			468			477			
GTC	TTC		CTG	GCA		TCĊ	TCC		AGC	ACC	TCT	GGG	GGC		GCG	GCC	
v	 F	 Р	L	 A	 P	 s	 S		 s	T	s	 G	 G	 T	 A	 A	
					E0.												
GGC	TGC	495 CTG	GTC	AAG	504 GAC	TAC	TTC	513 CCC	GAA	CCG	522 GTG	ACG	GTG	531 TCG	TGG	AAC	
G	С	ь	٧	K		Y	F	P	E	P	V	T	v	s	W	N	
GGC	GCC	549 CTG	ACC	AGC	558 GGC	GTG	CAC	567 ACC	<b>T</b> ፓር	CCG	576 GCT	GTC	ርጥል	585 CAG	ጥርር	TCA	
															·		
G	A	L	T	s	G	V	Н	T	F	P	A	V	L	Q	5	s	
		603			612			621			630			639			,
	TAC		CTC	AGC	AGC	GTG	GTG	ACC	GTG	ccc	TCC	AGC	AGC	TTG	GGC	ACC	
L	Y	s	Ĺ	s	s	v	V	T	V	P	s	s	s	L	G	T	
		657			666			675			684			693			

Fig. 9(B) (Sheet 1 of 4)

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSNTKVD 729 747 720 738 711 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT V E P K S C D K T H T C P P C P A P 792 . 765 . 774 783 801 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT 828 837 846 855 819 CTC ATG ATC TCC CGG ACC, CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC L M I S R T P E V T C V V D V S H 909 . 900 882 891 873 918 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT E D P E V K F N W Y V D G V E V H N 936 945 954 963 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC 999 1017 1026 981 990 1008 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N G K E Y K C K 1044 1053 1035 1062 1071 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L P A P I E K T I S K A K 1107 1116 1089 1098 1125 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG --- --- --- --- --- --- --- --- --- --- --- --- --- ---G Q P R E P Q V .Y T L P P S R D E L 1161 1170 1179 1152 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC T K N Q V S L T C L V K G F Y P S D 1197 1206 1215 1224 1233 1242 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG 1251 1260 1269 1278 1287 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDGSFFLYSKLTV 1323. 1332 1314 1341 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG D K S R WQQGNVFSCSVMHE

Fig. 9(B) (Sheet 2 of 4)

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							J	"/ <b>I</b>	T	,							
CCT		1359	A A C		1368			1377			1386			1395			1404 GGC
A	L	H	N	н	Y	T	Q	,K	S	.T	s	L	s	P	<u></u>	s	G
		1413			1422	mmo		1431			1440			1449			1458
GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA			GAG	ACC	AAG	A1G	TCC
_ <u>G</u>	, L	K	1	A	A	F	N	I	Q	T	F	G	E	T	K	М	s
		1467			1476			1485			1494			1503		*	1512
TAA	GCC	ACC	CIC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	CTG	AGC	CGC	TAC	GAC	ATC	GCC
N	λ	T	L	Ÿ	s	Y	I	v	Q	I	L	s	R	Y	D	I	λ
	:	1521			1530		:	1539			1548			1557			1566
CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG	AAG	CTG	CTG	GAC
L	v	Q	E	v	R	D	s	H	L	T	A	v	Ġ	ĸ	L	L	D
	:	1575		:	1584			1593		:	1602			1611			1620
AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GTG	GTC	AGT	GAG		CTG
, N	L	N	Q	Ð	A	P	D	·T	Y	H	Y	v	v	s	E		L
		1629			1638			1647			1656			1665			1674
GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG	TAC	AGG	CCT	GAC	CAG	GTG
G	R	N	s	Y	ĸ	E	R	Y	L	F	v	Y	R	P	D	Q	v
		1683			1692			1701			1710		:	1719		:	1728
TCT			GAC			TAC			GAT	GGC	TGC	GAG	CCC	TGC	GGG		
s	 A	v	D	s	Y	Y	Y	D	D	G	c	E	P	С	G	N	D
	:	1737			1746			1755			1764		:	1773		:	1782
ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT		AGG	TTC	TTC	TCC	ÇGG	TTC	ACA	GAG	GTC
T	F	N	R	E	P	A	1	v	R	F	F	s	R	F	T	E	v
	:	1791			1800			1809			1:818			1827		3	1836
AGG			GCC											GCA			
R	E	F	A	1	v	P	L	н	A	A	P	G	D	A	v	A	E
		1845		:	1854			1863			1872	•		1881		1	1890
ATC			CTC			GTC			GAT			GAG		TGG	GGC		
I	D	A	L	Y	D	v	Y	L	D	v	ġ	E	ĸ	W	G	L	Ē
		1899			1908		1	.917		1	1926			1935		I	1944
			TTG	ATG	GGC	GAC	TTC	TAA	GCG	GGC	TGC			GTG	AGA	ccc	TCC
D			L		 G		 F		A		c		Y	. v	R	P	\$
		1953		:	1962		,	1971			1980		:	1989		. 1	1998
CAG			TCC	ATC	CGC	CTG			AGC			TTC		TGG	CTG		
Q	w	s	s	I	R	t	w	т	s	P	. T	F	Q	W	L	I	P
		2007			2016			2025		1	2034			2043			2052
GAC .			GAC			GCT			ACG			GCC		GAC			
D	s	A		T		_A			т	н	С	Α	Y	D	R	I	v
		•	$\boldsymbol{F}$	iρ	. 9	90	B)	)									
									`\								
		•	(D	n (	eei	<i>3</i>	<i>vj</i>	4	•								

	:	2061			2070		:	2079		:	2088		:	2097			2106
GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GĊC	GTT	GTT	CCC	GAC	TCG	GCT	CTT	CCC	TTT
									<i>-</i>								
v	A	G	М	L	L	R	G	A	v	V	P	Û	s	A	L	₽	F
	٠,	2115			2124			2133		,	2142		-	2151			2160
										-							
AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC	AGT	GAC
			<b></b>														
N	F	Q	Α.	A	Y	G	L	s	D	. Q	L	A	Q	A	I	s	D
							_				•						
	- 4	2169		- 4	2178		7	2187									
CAC	TAT	CCA	GTG	GAG	GTG	ATG	CTG	AAG	TGA	3′							
H	Y	P	v	E	v	M	L	K	*								

Fig. 9(B) (Sheet 4 of 4)

### 37/113 pAS37

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2196 bp 2196 bp DNA
                                                                  14-AUG-
                         2226 bp
           PAS37. DNA
LOCUS
1998
DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse construct 37
DEFINITION Clone 16.4.2 (same as hcdnasel.dna template file) plus NLS
REFERENCE
           VERHOEYEN ET AL
 AUTHORS
           CONSTRUCTION OF RESHAPED HMFG1 etc
  TITLE
           IMMUNOL. (1993):78, 364-370
  JOURNAL
           Human DNAse sequence is modified as a result of oligo assembly
COMMENT
(mhdnase.dna)
           The fusion was made using overlapping oligos AS79 and AS80
COMMENT
           AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES
            Residue 963 is G > T leading to silent mutation in all clones
FEATURES
  SITES
            Note
                                           413 t
                         683 c
                                  619 q
BASE COUNT
                511 a
                 ?
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
      781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
      841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
      901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
      961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
     1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
     1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
     1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
     1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
     1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
     1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
     1381 CTCTCCCTGT CTCCGGGTAA AGGGAGCGGC GGGCTGAAGA TCGCAGCCTT CAACATCCAG
     1441 ACATTTGGGG AGACCAAGAT GTCCAATGCC ACCCTCGTCA GCTACATTGT GCAGATCCTG
     1501 AGCCGCTACG ACATCGCCCT GGTCCAGGAG GTCAGAGACA GCCACCTGAC TGCCGTGGGG
     1561 AAGCTGCTGG ACAACCTCAA TCAGGACGCA CCAGACACCT ATCACTACGT GGTCAGTGAG
     1621 CCACTGGGAC GGAACAGCTA TAAGGAGCGC TACCTGTTCG TGTACAGGCC TGACCAGGTG
     1681 TCTGCGGTGG ACAGCTACTA CTACGATGAT GGCTGCGAGC CCTGCGGGAA CGACACCTTC
     1741 AACCGAGAGC CAGCCATTGT CAGGTTCTTC TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC
     1801 ATTGTTCCCC TGCATGCGGC CCCGGGGGAC GCAGTAGCCG AGATCGACGC TCTCTATGAC
     1861 GTCTACCTGG ATGTCCAAGA GAAATGGGGC TTGGAGGACG TCATGTTGAT GGGCGACTTC
     1921 AATGCGGGCT GCAGCTATGT GAGACCCTCC CAGTGGTCAT CCATCCGCCT GTGGACAAGC
     1981 CCCACCTTCC AGTGGCTGAT CCCCGACAGC GCTGACACCA CAGCTACACC CACGCACTGT
     2041 GCCTATGACA GGATCGTGGT TGCAGGGATG CTGCTCCGAG GGGCCGTTGT TCCCGACTCG
     2101 GCTCTTCCCT TTAACTTCCA GGCTGCCTAT GGCCTGAGTG ACCAACTGGC CCAAGCCATC
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2221 GTTTGA

2161 AGTGACCACT ATCCAGTGGA GGTGATGCTG AAGGGGGGGC GACCCAAAAA GAAGCGCAAG

		9			10			22			36			4 5			
ATG	GGA	-	AGC	TGT	18 ATC		CTC	27 TTC		GTA			GCT	45 ACA		GTC	CAC
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		171			180			189			198			207			21.6
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		.225			234			243			252			261			270
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		279			288			297			306			315			324
AGA	GAC		TCC	ACA		ACA	GCC		ATG	GAG		AGC	AGC		AGG	TCT	
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GAC .	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
D	T	A	v	Y	Y	С	A	R	s	Y	D	F	A	W	F	Α.	Y
		387			396			405			414			423			432
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	
W	G	Q	G	Т	L	v	T	V	S	s	A	S	T	K	G	P	s
		441			450	•		459			468			477			486
GTC	TTC	ccc	CTG	GCA	ccc	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC			GĊC	CTC
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Fig. 10(B) (Sheet 1 of 4)

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ACC	TAC	ATC	TGÇ	AAC	GTG	TAA	CAC	AAG	ccc	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA 
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		711			720			729			738			747			756
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT
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GAA	CTC	CTG	GGG	GGA	CCG	TCA	GIC	TTC	CTC	TTC		CCA	AAA		AAG	GAC	ACC
E	L	L	G	G	P	s	v	F	L	F	P	P	K	P	ĸ	D	T
		819			828			837		٠	846			855			864
CIC	ATG	ATC	TCC	CGG	ACC	CCT	GAG	GTC	ACA	TGC	GTG	GTG	GTG	GAC	GTG	AGC	CAC
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		873			882			891			900			909			918
GAA	GAC	CCT	GAG	GTC	AAG	TTC	AAC	TGG	TAC	GTG	GAC	GGC	GTG	GAG	GTG	CAT	TAA
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		927			936			945			954			963			972
GCC	AAG		AAG	CCG		GAG	GAG		TAC	AAC		ACG	TAC		GTG	GTC	
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		1035			1044			1053			1062			1071			L080
GTC	TCC	AAC	AAA	GCC	CTC	CCA	GCC	ccc	ATC	GAG	AAA	ACC	ATC	TCC	AAA	GCC	AAA
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		1143			1152			1161			1170			1179			1188
ACC	AAG	AAC	CAG	GTC	AGC	CTG	ACC	TGC	CTG	GTC	AAA	GGC	TIC	TAT	ccc	AGC	GAC
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		1197			1206			1215						1233			1242
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		1251			1260			1269							СТС		1296. GTG
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GAC	AAG	AGC	AGG		CAG		GGG		GTC	TTC					ATG		
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Fig. 10(B) (Sheet 2 of 4)

## 40/113

	:	L359			1368			1377			1386			1395			1404
GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCT	CCC	GGT	AAA	GGC
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AGC	GGC	GGG	CTG	AAG	ATC	GÇA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG	ACC	AAG
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	, :	1467			1476			1485			1494			1503			1512
atg	TCC	TAA	GCC	ACC	CTC	GTC	AGC	TAC	TTA	GTG	CAG	ATC	CTG	AGC	CGC	TAC	GAC
М	S	N	A	T	L	v	s.	Y	1	v	Q	1	L	s	R	Y	D
		1521			1530			1539			1548			1557			1566
ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG	AAG	CTG
I	A	L	v	Q	E	V	R	D	S	H	L	T	A	V	G.	K	L
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		1575			1584			1593			1602			1611			1620
CIG	GAC	AAC	CIC	AAT	CAG	GAÇ	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GTG	GTC	AGT	GAG
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CCA			ന്ദര		AGC	тат			CGC			TTC			¥GG		
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	. 1	1683		:	1692		1	L701		1	1710		:	1719		1	1728
CAG	GTG	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT	GGC	TGC	GAG	CCC	TGC	GGG
Q	v	s	A	v	D	s	Y	Y	Y	D	D	G	С	E	P	С	G
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	1	1737		:	1746		1	1755		1	L764		1	L773		1	1782
AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	TTA	GTC	AGG	TTC	TIC	TCC	CGG	TTC	ACA
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		1791			1800			1809			1818			1827			1836
GAG	GTC	AGG	GAG	TTT	GCC	TTA	GTT	CCC	CTG	CAT	GCG	GCC	CCG	GGG	GAC	GCA	GTA
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ATC					GAC							CAC	IGT	GCC	TAT	GAC	AGG
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Fig. 10(B) (Sheet 3 of 4)

	7	2061		2	2070		2	2079		:	8809		2	2097		2	2106
ATC	GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	GAC	TCG	GCT	CTT
I	v	V	A	G	M	L	L	R	G	A	V	V	P	D	S	A	ւ
		2115			2124						2142			2151			2160
CCC	TTT	AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC
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		2169		•	2178			2187			2196		:	2205		:	2214
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AGT			TAT		2178 GTG				CTG						AAA		
	GAC	CAC		CCA	GTG	GAG	GTG	ATG				GGC	GGA	ccc	AAA  K_		
AGT 			TAT			GAG		ATG		AAG	GGG	GGC		ccc	AAA K		
	GAC  D	CAC		CCA	GTG	GAG	GTG	ATG		AAG	GGG	GGC	GGA	ccc	AAA K		
s	GAC D	CAC  H 2223		CCA  P	GTG	GAG	GTG	ATG		AAG	GGG	GGC	GGA	ccc	AAA K		
s	GAC D	CAC  H 2223	Y	CCA  P	GTG	GAG	GTG	ATG		AAG	GGG	GGC	GGA	ccc	AAA K		

Fig. 10(B) (Sheet 4 of 4)

### 42/113 pAS38

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2223 bp
                                   2193 bp
LOCUS
            PAS38, DNA
                                               DNA
                                                               14-AUG-1998
            HUMANISED HMFG1 heavy chain fused to human DNAse construct 38
DEFINITION
            Clone 17.12.1 with silent K to K mutation (1398 A > G)+NLS
DEFINITION
REFERENCE
  AUTHORS
            VERHOEYEN ET AL
            CONSTRUCTION OF RESHAPED HMFG1 etc
  TITLE
            IMMUNOL. (1993):78, 364-370
  JOURNAL
            Human DNAse sequence is modified as a result of oligo assembly
COMMENT
(mhdnase.dna)
            The fusion was made using overlapping oligos AS81 and AS82
COMMENT
FEATURES
            AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
            Residue 963 is G > T leading to silent mutation in all clones
FEATURES
FEATURES
            In 17.12.1 residue 1398 is A > G (silent K to K mutation)
  SITES
            Note
BASE COUNT
                510 a
                         683 c
                                  618 g
                                           412 t
ORIGIN
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1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG 61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC 121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA 181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT 241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG 301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC 361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC 421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG 481 GCCCTGGCCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA 541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC 601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC 661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT 721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC 781 TTCCTCTTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA 841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC 901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC 961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG 1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA 1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG 1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG 1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC 1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG 1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC 1381 CTCTCCCTGT CTCCGAAg<u>eg GAGCGGCGGG</u> CTGAAGATCG CAGCCTTCAA CATCCAGACA 1441 TTTGGGGAGA CCAAGATGTC CAATGCCACC CTCGTCAGCT ACATTGTGCA GATCCTGAGC 1501 CGCTACGACA TCGCCCTGGT CCAGGAGGTC AGAGACAGCC ACCTGACTGC CGTGGGGAAG 1561 CTGCTGGACA ACCTCAATCA GGACGCACCA GACACCTATC ACTACGTGGT CAGTGAGCCA 1621 CTGGGACGGA ACAGCTATAA GGAGCGCTAC CTGTTCGTGT ACAGGCCTGA CCAGGTGTCT 1681 GCGGTGGACA GCTACTACTA CGATGATGGC TGCGAGCCCT GCGGGAACGA CACCTTCAAC 1741 CGAGAGCCAG CCATTGTCAG GTTCTTCTCC CGGTTCACAG AGGTCAGGGA GTTTGCCATT 1801 GTTCCCCTGC ATGCGGCCCC GGGGGACGCA GTAGCCGAGA TCGACGCTCT CTATGACGTC 1861 TACCTGGATG TCCAAGAGAA ATGGGGCTTG GAGGACGTCA TGTTGATGGG CGACTTCAAT 1921 GCGGGCTGCA GCTATGTGAG ACCCTCCCAG TGGTCATCCA TCCGCCTGTG GACAAGCCCC 1981 ACCTTCCAGT GGCTGATCCC CGACAGCGCT GACACCACAG CTACACCCAC GCACTGTGCC 2041 TATGACAGGA TCGTGGTTGC AGGGATGCTG CTCCGAGGGG CCGTTGTTCC CGACTCGGCT 2101 CTTCCCTTTA ACTTCCAGGC TGCCTATGGC CTGAGTGACC AACTGGCCCA AGCCATCAGT 2161 GACCACTATC CAGTGGAGGT GATGCTGAAG GGGGGCGGAC CCAAAAAGAA GCGCAAGGTT 2221 TGA

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	GTG	AAG	GTG	TCC	TGC	126 AAG	GCT	TCT	GGC	TAC	ACC	144 TTC	AGT	GCC	153 TAC	TGG	ATA	162 GAG
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			387			396			405			414			423			432
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	v	T	v	s	s	A	s	Ŧ.	K	G	P	s
	GTC	TTC	441 CCC	CTG	GCA	450 CCC		TCC		AGC	ACC	468 TCT		GGC	477 ACA	GCG	GCC	486 CTG
						 P	 s	 s	 K	 s		 s		 G		 A	 A	
	. <b>v</b>	F	P	L	A	r	3	3	K	3			G	•		•	•	
	GGC	TGC	495 CTG	GTC	AAG	504 GAC	TAC	TTC	513 CCC	GAA	CCG	522 GTG	ACG	GTG	531 TCG	TGG	AAC	540 TCA
																		·
	G	С	L	V	K	D	Y	F	₽	E	P	V	T	V	s	W.	N	s
			549									576					<b>003</b>	594
	GGC	GCC	CTG	ACC	AGC	GGC		CAC		TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA.	GGA
	G	A	L	T	s	G	v	H	T	F	P	A	v	L	Q	s	s	G.
			603			612									639			648
	CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GIG		TCC		AGC	TTG	GGC	ACC	CAG
	L	Y	5	L	s	s	v	V	т	v				s	L	G	т	Q
			657			666			675			684			693		•	702

Fig. 11(B) (Sheet 1 of 4)

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA TYICNVNHKPSNTKVDKK . 720 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC LMISRTPEVTCVVVDVSH GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT EDPEVKFNWYVDGVEVHN GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC AKTKPREEQYNST,YRVVS GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---V L T V L H Q D W L N G K E Y K C K GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA V S N K A L · P A P I E K T I S K A K GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG GQPREPQVYTLPPSRDEL ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC T K N Q V S L T C L V K G F Y P S D ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG I. A V E W E S N G Q P E N N Y K T T CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDG, SFFLYSKLTV · 1332 .1305 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG D K S R W Q Q G N V F S C S V M H E

Fig. 11(B) (Sheet 2 of 4)

1395 1404 GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCG AAG GGG AGC ALHNHYTQKSLSPK<u>GS</u> GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT GGG GAG ACC AAG ATG GGLKIAAFNIQTFGETKM 1476 · 1485 TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG AGC CGC TAC GAC ATC S N A T L V S Y I V Q I L S R Y D I GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC GTG GTC AGT GAG CCA D N L N Q D A P D T Y H Y V V S E P CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG 1692 1701 1710 GTG TCT GCG GTG GAC AGC TAC TAC TAC GAT GAT GGC TGC GAG CCC TGC GGG AAC V S A V D S Y Y Y D D G C E P C G N 1773 1782 GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC TCC CGG TTC ACA GAG D T F N R E P A I V R F F S R F T E 1827 . GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG GGG GAC GCA GTA GCC V R E F A I V P L H A A P G D A V A GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT GTC CAA GAG AAA TGG GGC TTG --- --- --- --- --- --- --- --- ---E I D A L Y D V Y L D V Q E K W G L GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG GGC TGC AGC TAT GTG AGA CCC EDVMLMGDFNAGCSYVRP TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC CCC ACC TTC CAG TGG CTG ATC S Q W S S .I R L W T S P T F Q .W L I CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG CAC TGT GCC TAT GAC AGG ATC P D S A D T T A T P T H C A Y D R I

Fig. 11(C) (Sheet 3 of 4)

	2061 2070 2079 IG GTT GCA GGG ATG CTG CTC CGA GGG						2079		2000							2106	
GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	GAC	TCG	GCT	CTT	CCC
											·						
v	V	Α	G	M	L	L	R	G	A	V	V	P	D	s	A	L	P
													•				
	:	2115			2124			2133		:	2142		:	2151		:	2160
TŢŢ	AAC	TTC	CAG	GCT	GCC	TAT	GGÇ	CTG	AGT	GAC	CAA	CTG	GCC	CAA	GCC	ATC	AGT
F	N	F	Q	A	A	Y	٠G	L-	s	D	Q	L	A	Q	Α	I	s
	:	2169		:	2178		:	2187		:	2196		:	2205		:	2214
GAC																AAG	
GAC																	
GAC  D	CAC		CCA				ATG				GGC		ccc	AAA 			
	CAC	TAT	CCA	GTG 	GAG	GTG 	ATG	CTG	AAG	GGG	GGC	GGA	ccc	AAA 	AAG		CGC
	CAC H	TAT	CCA	GTG 	GAG	GTG 	ATG	CTG	AAG	GGG	GGC	GGA	ccc	AAA 	AAG		CGC
Đ	CAC H	TAT  Y	CCA P	GTG 	GAG	GTG 	ATG	CTG	AAG	GGG	GGC	GGA	ccc	AAA 	AAG		CGC
Đ	CAC H	TAT  Y 2223	CCA P	GTG 	GAG	GTG 	ATG	CTG	AAG	GGG	GGC	GGA	ccc	AAA 	AAG		CGC
Đ	CAC H GTT	TAT  Y 2223	CCA P	GTG 	GAG	GTG 	ATG	CTG	AAG	GGG	GGC	GGA	ccc	AAA 	AAG		CGC

Fig. 11(D) (Sheet 4 of 4)

### 47/113 pAS39

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14-AUG-
                        2220 bp 2190 bp
                                             DNA
           PAS39.DNA
LOCUS
1998
DEFINITION HUMANISED HMFG1 heavy chain fused to human DNAse - construct 39
DEFINITION Clone 18.24.1 with residue 1392 T > C +NLS
REFERENCE
            VERHOEYEN ET AL
  AUTHORS
            CONSTRUCTION OF RESHAPED HMFG1 etc
  TITLE
            IMMUNOL. (1993):78, 364-370
  JOURNAL
            Human DNAse sequence is modified as a result of oligo assembly
COMMENT
(mhdnase.dna)
            The fusion was made using overlapping oligos AS83 and AS84
COMMENT
            AA RESIDUE 235 HAS NOT BEEN CHANGED TO KABAT (I.E. V TO A)
FEATURES
            Residue 963 is G > T leading to silent mutation in all clones
FEATURES
            Residue 1392 T > C silent S to S mutation
FEATURES
            Note
  SITES
                                           411 t
                                  617 g
                508 a
                         684 c
BASE COUNT
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC
      781 TTCCTCTCC CCCCAAAACC CAAGGACACC CTCATGATCT CCCGGACCCC TGAGGTCACA
      841 TGCGTGGTGG TGGACGTGAG CCACGAAGAC CCTGAGGTCA AGTTCAACTG GTACGTGGAC
      901 GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC
      961 CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CAGGACTGGC TGAATGGCAA GGAGTACAAG
     1021 TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA
     1081 GGGCAGCCCC GAGAACCACA GGTGTACACC CTGCCCCCAT CCCGGGATGA GCTGACCAAG
     1141 AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT CGCCGTGGAG
     1201 TGGGAGAGCA ATGGGCAGCC GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC
     1261 GACGGCTCCT TCTTCCTCTA CAGCAAGCTC ACCGTGGACA AGAGCAGGTG GCAGCAGGGG
     1321 AACGTCTTCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC GCAGAAGAGC
     1381 CTCTCCCTGT CcCCGGGGGC CGGCGGGCTG AAGATCGCAG CCTTCAACAT CCAGACATTT
     1441 GGGGAGACCA AGATGTCCAA TGCCACCCTC GTCAGCTACA TTGTGCAGAT CCTGAGCCGC
     1501 TACGACATCG CCCTGGTCCA GGAGGTCAGA GACAGCCACC TGACTGCCGT GGGGAAGCTG
     1561 CTGGACAACC TCAATCAGGA CGCACCAGAC ACCTATCACT ACGTGGTCAG TGAGCCACTG
     1621 GGACGGAACA GCTATAAGGA GCGCTACCTG TTCGTGTACA GGCCTGACCA GGTGTCTGCG
     1681 GTGGACAGCT ACTACTACGA TGATGGCTGC GAGCCCTGCG GGAACGACAC CTTCAACCGA
     1741 GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG TTCACAGAGG TCAGGGAGTT TGCCATTGTT
     1801 CCCCTGCATG CGGCCCCGGG GGACGCAGTA GCCGAGATCG ACGCTCTCTA TGACGTCTAC
     1861 CTGGATGTCC AAGAGAAATG GGGCTTGGAG GACGTCATGT TGATGGGCGA CTTCAATGCG
     1921 GGCTGCAGCT ATGTGAGACC CTCCCAGTGG TCATCCATCC GCCTGTGGAC AAGCCCCACC
     1981 TTCCAGTGGC TGATCCCCGA CAGCGCTGAC ACCACAGCTA CACCCACGCA CTGTGCCTAT
     2041 GACAGGATCG TGGTTGCAGG GATGCTGCTC CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT
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2101 CCCTTTAACT TCCAGGCTGC CTATGGCCTG AGTGACCAAC TGGCCCAAGC CATCAGTGAC 2161 CACTATCCAG TGGAGGTGAT GCTGAAGGGG GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

11

18 -36 5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC 90 72 81 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA <u>S</u>QVQLVQSGAEVK PGAS 126 135 144 GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG V K V S C K A S G Y T F S A Y W I E 171 180 189 198 TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT W V R Q A P G K G L E W V G E I L P 252 · 243 234 261 225 . . GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT G S N N S R Y N E K F K G R V T V T 297 . 306 288 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG R D T S T N T A Y M E L S S L R S E 333 342 351 360 369 GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC D T A V Y Y C A R S Y D F A W F A Y 387 396 405 414 423 TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG 450 459 468 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG 531 · 522 504 513 495 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA G C L V K D Y F P E P V T V S W N S 567 576 . GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA 603 612 621 630 639 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG L Y S L S S V V .T V P S S S L G T Q 657 666 675 684 702

Fig. 12(B) (Sheet 1 of 4)

54 45 36 5' ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG GTA GCA ACA GCT ACA GGT GTC CAC G W S C I I L F L V A T A T G V H 72 90 81 TCC CAG GTG CAG CTG GTG CAG TCT GGG GCA GAG GTG AAA AAG CCT GGG GCC TCA S Q V Q L V Q S G A E V K K P G A S 153 126 135 144 GTG AAG GTG TCC TGC AAG GCT TCT GGC TAC ACC TTC AGT GCC TAC TGG ATA GAG V K V S C K A S G Y T F S A Y W I E 171 180 189 198 TGG GTG CGC CAG GCT CCA GGA AAG GGC CTC GAG TGG GTC GGA GAG ATT TTA CCT \_\_\_ \_\_\_ \_\_\_ \_\_\_ W V R Q A P G K G L E W V G E I L P 234 225 243 252 261 GGA AGT AAT AAT TCT AGA TAC AAT GAG AAG TTC AAG GGC CGA GTG ACA GTC ACT G S N N S R Y N E K F K G R V T V T 315 297. 306 279 288 AGA GAC ACA TCC ACA AAC ACA GCC TAC ATG GAG CTC AGC AGC CTG AGG TCT GAG RDTSTNTAYMELSSLRSE 342 351 360 GAC ACA GCC GTC TAT TAC TGT GCA AGA TCC TAC GAC TTT GCC TGG TTT GCT TAC D T A V Y Y C A R S Y D F A W F A Y 405 423 387 396 414 TGG GGC CAA GGG ACT CTG GTC ACA GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG W G'Q"G T L V T V S S A S T K G P S 459 468 450 GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG V F P L A P S S K S T S G G T A A L 504 513 522 531 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA G C L V K D Y F P E P V T V S W N S 558 . 567 576 585 GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA G A L T S G V H T F P A V L Q S S G 621 630 612 639 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG L Y S L S S V V T V P S S S L G T Q 666 675 684 657 693 702

Fig. 12(B) (Sheet 1 of 4)

ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA 729 720 738 747 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC-CCA CCG TGC CCA GCA CCT V E P K S C D K T H T C P P C P A P 765 774 783 792 810 801 GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC ELLGGPSVFLFPPKPKDT 837 819 828 846 CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG GAC GTG AGC CAC --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---LMISRTPEVTCVVDVSH 882 891 900 GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT AAT E D P E V K F N W Y V D G V E V H N 936 · 945 963 954 GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC A K T K P R E E Q Y N S T Y R V V S 1008 990 999 1026 1017 GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG V L T V L H Q D W L N. G K E Y K C K 1071 1080 1044 1053 1062 1035 GTC TCC AAC AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---V S N K A L P A P I E K T I S K A K 1098 1107 1116 1125 GGG CAG CCC CGA GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG --- --- --- --- --- --- --- --- --- --- --- --- --- ---G Q P R E P Q V Y T L P P S R D E L 1152 1161 1170 ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC T K N Q V S L T C L V K G F Y P S D 1206 1215 1233 1224 1242 ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG I A V E W E S N G Q P E N N Y K T T 1251 1260 1269 1278 CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AAG CTC ACC GTG PPVLDSDGSFFLYSKLTV 1341 1350 1305 1314 1332 1323 GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC TCC GTG ATG CAT GAG D K S R W Q Q G N V F S C S V M H E

Fig. 12(B) (Sheet 2 of 4)

		1	1359		1	368		1	377		1	1386		1	1395		1	1404	
	GCT	CTG	CAC	AAC	CAC	TAC	ACG	CAG	AAG	AGC	CTC	TCC	CTG	TCC	CCG	GGG	AGC	GGC	
			11	NT.	ш	v	æ	0	ĸ	c	f.	S	τ.	S	P	G	s	G	
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					_				433			440		-	1449		1	450	
		3	1413		1	422		1	.431	•		1440							
	CCC.	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG	ACC	AAG	ATG	TCC	
	G	L	ĸ	I	A	Α	F	N	I	Q	T	F	G	E	T	ĸ	М	s	
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			1467		3	476		1	485			1494		1	L503		1	1512	
	3 A M		ACC.													GAC	ATC	GCC	
	AA1	GCC	ACC	CIC	GIC	AGC	IAC												
												·			v	-			
	N	A	T	L.	V	S	Y	1	V	Q	1	L	5	ĸ	1	D	1	A	
		:	1521		1	.530		1	L <b>539</b>			1548			L557		- ]	L566	
	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG	ACT	GCC	GTG	GGG	AAG	CTG	CTG	GAC	
	τ.	v	٥	E	v	R	D	s	H	L	T	A	V	G	K	L	L	Ø	
	-	•	¥	_	•	••	•	_		_									
			1575			1594			1503			1602			1613			1620	
			T212			2004			200	m. m	030	my C	CIV.	CITC.	እርጥ	GAG	cos.	CITC	
	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT					WGI	GAG	CLA	C10	
	N	L	N	Q	D	A	P	D	T	Y	Н	¥	V	V	S	E	Р	L	
	1629 1638 1647 1656 1665 1 GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG TAC AGG CCT GAC CAG															1674			
	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG	TAC	AGG	CCT	GAC	CAG	GTG	
•		<u></u>																	
	G	R	N	s	Y	K	E	R	Y	L	F	٧	Y	R	P	D	Q	v	
	1683 1692 1701 1710 1719 1															1728			
	•																		
	TCT	GCG	GIG	GMC	AGC	INC	INC	INC	GAL										
					s												N	D	
	S	A	V	ט	S	¥	Y	Y	ע	D	G	•		r	_	G	14	D	
															, , , ,				
			1737			1746		•	1/22			704			1773				
	ACC	TTC	AAC	CGA	GAG	CCA	GCC	TTA	GTC	AGG	TTC	TTC	TCC	CGG	TTC	ACA	GAG	GTC	
	T	F	N	R	E	P	A	I	v	R	F	F	S	R	F	Ť	E	v	
													•						
			1791			1800		. :	1809			1818			1827		:	1836	
	AGG	GAG	TTT	GCC	ATT	GTT	CCC	CTG	CAT	GCG	GCC	CCG	GGG	GAC	GÇA	GTA	GCC	GAG	
																-:-			
				Δ	т	v	P	۲.	н	A	A	P	G	D	A	v	A	E	
	•		•	••	-	•	_	_	••		•								
			1845			105/			1863			1872			1881			1890	
		~	TO42		m>0	T024	CID.	mac.	CTC		CITY	C D D	242	444		GGC			
	ATC	GAC	GCT	CIC	TAT	GAC	GIC				4.0								
													-	v	W	G			
	I	D	A	L	Y	D	V	Y	ь	D	٧	v	£	K	**	G	L	E	
			1899			1908			1917			1926			1935			1944	
	GAC	GTC	ATG	TTG	ATG	GGC	GAC	TTC	TAA	GCG	GGC	TGC	AGC	TAT	GTG	AGA	CCC	TCC	
	D	v	М	L	M	G	D	F	N	A	G	С	S	Y	V	R	₽	s	
			1953			1962			1971			1980			1989		- 1	1998	
	CVC	TCC													. TGG	CTG	ATC	CCC	
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						2026			2025			2034			2043			2052	
			2007			2016			2025										
	GAC	AGC	GCI	GAC	ACC			ACA	CCC	ACG	CAC	161	GUU	IAT	GAC	AGG	ATC	GTG	
	D	s	A	D	T	T	A	T	. P	T	Н	С	A	Y	D	R	I	V	
						•	D:	~	1	2/	D	1							
							Fi	Υ.	I.	4(	D	/							

Fig. 12(B)
(Sheet 3 of 4)
SUBSTITUTE SHEET (RULE 26)

		2061		:	2070		2	2079		;	2088		;	2097		:	2106
CTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	GAC	TCG	GCT	CTT	CCC	TTT
ν	A	G	М	Ĺ	L	R	G	A	V	V	Ą	D	s	A	L	P	F
	2115 2124 TTC CAG GCT GCC TAT (						-	2133			2142			2151			2160
AAC	TIC	CAG	GCT	CCC	TAT	CCC	CIG	AGT	GAC	CAA	CIG	GCC	CAA	GCC	ATC	AGT	GAC
N	F	Q	A	A	Y	G	L	s	D	Q	L	A	Q	A	I	s	D
										•							
	:	2169		2	2178		2	2187		2	2196		7	205		:	2214
CAC	TAT	CCA	GTG	GAG	GTG	atg	CTG	AAG	GGG	GGC	GGA	ccc	AAA	AAG	AAG	CGC	AAG
H	Y	p	V	E	V	M	L	K	G	G	G	P	ĸ	ĸ	K	R	ĸ

GTT TGA 3

Fig. 12(B) (Sheet 4 of 4)

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# 52/113 pAS101

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mRNA
                                                               06-MAR-1995
                                                    PRI
            PAS101.DNA
                         1548 bp
LOCUS
DEFINITION Humanised HMFG1 Fab 2 fused to human DNase I (pAS101)
ACCESSION
NID
KEYWORDS
            DNase I.
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
MHDNASE1.dna)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic
  TITLE
fibrosis
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
            91067672
  MEDLINE
                                  430 g
                                           308 t
                         467 c
                343 a
BASE COUNT
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCCTGAA GATCGCAGCC
      781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
      841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
      901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
      961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
     1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
     1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
     1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
      1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
     1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
      1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
      1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
      1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
      1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA
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```
FDDNASE101 1548 BP SS-DNA
LOCUS
                                                    SYN
                                                              25-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
FEATURES
                    Location/Qualifiers
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     frag
                     /note="1 to 1548 of PAS101.dna [Split]"
     frag
                     721..780
                     /note="1 to 60 of 101/105linker"
                     join(721...>735,<736...>759,<760...>780)
     frag
                     /note="1 to 80 of 102linker [Split]"
BASE COUNT
                343 A
                        465 C
                                  431 G
                                           309 T
ORIGIN
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       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
     721 GACAAAACTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GCGGGCTGAA GATCGCAGCC
     781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
      841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
      901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
      961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
     1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
     1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
     1141 AGGGAGTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
     1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
    1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
     1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
     1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
     1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
    1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGTGA
```

Fig. 13(B)

```
SYN
                                                              29-AUG-2000
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LOCUS
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
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                     join(10..>729,<790..1557)
     frag
                     /note="1 to 1548 of PAS101.dna [Split]"
     fraq
                     /note="1 to 60 of 101/105linker"
                    join(730..>744,<745..>768,<769..>789)
     frag
                    /note="1 to 80 of 102linker [Split]"
                                           309 T
                         471 C
                                  433 G
BASE COUNT
                344 A
ORIGIN
        1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
      181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
      301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
      361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
      481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
      541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
      661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
      721 AAATCTTGTG ACAAAACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG CGGGCTGAAG
      781 ATCGCAGCCT TCAACATCCA GACATTTGGG GAGACCAAGA TGTCCAATGC CACCCTCGTC
      841 AGCTACATTG TGCAGATCCT GAGCCGCTAC GACATCGCCC TGGTCCAGGA GGTCAGAGAC
      901 AGCCACCTGA CTGCCGTGGG GAAGCTGCTG GACAACCTCA ATCAGGACGC ACCAGACACC
      961 TATCACTACG TGGTCAGTGA GCCACTGGGA CGGAACAGCT ATAAGGAGCG CTACCTGTTC
     1021 GTGTACAGGC CTGACCAGGT GTCTGCGGTG GACAGCTACT ACTACGATGA TGGCTGCGAG
     1081 CCCTGCGGGA ACGACACCTT CAACCGAGAG CCAGCCATTG TCAGGTTCTT CTCCCGGTTC
     1141 ACAGAGGTCA GGGAGTTTGC CATTGTTCCC CTGCATGCGG CCCCGGGGGA CGCAGTAGCC
     1201 GAGATCGACG CTCTCTATGA CGTCTACCTG GATGTCCAAG AGAAATGGGG CTTGGAGGAC
     1261 GTCATGTTGA TGGGCGACTT CAATGCGGGC TGCAGCTATG TGAGACCCTC CCAGTGGTCA
     1321 TCCATCCGCC TGTGGACAAG CCCCACCTTC CAGTGGCTGA TCCCCGACAG CGCTGACACC
     1381 ACAGCTACAC CCACGCACTG TGCCTATGAC AGGATCGTGG TTGCAGGGAT GCTGCTCCGA
     1441 GGGGCCGTTG TTCCCGACTC GGCTCTTCCC TTTAACTTCC AGGCTGCCTA TGGCCTGAGT
     1501 GACCAACTGG CCCAAGCCAT CAGTGACCAC TATCCAGTGG AGGTGATGCT GAAGTGA
11
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Fig. 13(C)

			. 9												45			
5′	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
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	n	G	**	3	_	-	•		•	,	v	^	•	-	٠	G	٠	
			63			72			8.1			90		٠	99			108
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT	GGG	GCA	GAG				ССТ	GGG	GCC	TCA
	S	Q	v	Q	L	v	Q	s	G	A	E	v	K	·K	P	G	A	S
			117	maa		126		<b>800</b>	135			144	» (~m	ccc	153			
	GIG	AAG	GIG	100	160	AAG	GCI	TCT		IAC	ACC					TGG	AIA	GAG
	v	ĸ	v	S	C	К	A	s	G	Y	T	F	s	A	<b>Y</b> .	W	I	E
	•			_	•	••		_		-							_	_
			171			180			189			198			207	•		216
	TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
	W	V	R	Q	A	P	G	K	G	L	E	W	v	G	E	I	L	P
			225			234			242			252			261			270
	GGA	AGT			ጥርጥ											ACA		
		-1-																
	G	s ·	N	N	s	R	Y	N	E	К	F	ĸ	G	R	v	T	v	T
			279			288			297			306						324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC.	ATG	GAG			AGC	CTG	AGG	TCT	GAG
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			333			342			351			360			369			378
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
	D	·T	A	V	Y	Y	С	A	R	s	Y	D	F	A	W	F	A	Y
			207			206			405			414			423			422
	TGG	GGC	387	ccc	ልርጥ	396 CTG										GGC		
	W	G	Q.	G	T	L	v	T	v	s	s	A	s	T	. к	G	P	s
	•									-								
			441			450			459			468						
	GTC	TTC	CCC	CTG												GCG		CTG
				т												Α		7.
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			495			504	,		513			522			531			540
	GGC	TGC	CTG	GTC	AAG	GAC	TAC	TTC	ccc	GAA	CĊG	GTG	ACG	GTG	TĊG	TGG	AAC	TCA
	G	С	L	V	K	D	Y	F	P	E	P	V	Т	V.	s	W	N	S
			549			558			567			576			585			594
	GGC	GCC			AGC			CAC								TCC		
	G	A	L	т	s	G	Ų	н	T	F	P	A	V.	L	Q	s	s	G

Fig. 13(D) (Sheet 1 of 3)

						•		<i>,</i>	L								
	603 612 621 630 639 648  CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG  L Y S L S S V V T V P S S S L G T Q																
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG.	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
t.	Y	s	L,	s	s	v	V	T	v	P	s	s	s	L	G	T	Q
	•	Ū	_	_	•					•							
		657			666			675			684			693			702
	m > C		T//	220		ጥፋል	CAC		ccc	<b>ACC</b>		ACC	AAG		GAC	AAG	
ACC	IAC	AIC	160	AAC	010	w	CAC	7.10									
							*1	К	P	s	N	T	K	v	D	к	ĸ
т	Y	I	С	N	V	N	H	ν.	P	3	14	•		•	Ü		
											77.0		•	747			756
		711			720			729			738					حفہ	
GTT	GAG	ccc	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	CCA	CCG	TGC	CCA	GCA	CCT
v	E	P	K	S	С	D	K	T	H	T	С	P	₽	С	P	A	₽
	•																
		765			774			783			792			801			810
GAA	GGC	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT	GGG	GAG	ACC	AAG
																	~
E	G	G	L	K	I	A	A	F	N	I	Q	T	F	G	E	T	K
		819			828			837			846			855			864
ATG	TCC		GCC	ACC		GTĊ	AGC	TAC	TTA	GTG	CAG	ATC	CTG	AGC	CGC	TAC	GAC
м	c	N	A	T	L	v	s	Y	I	v	Q	I	L	s	R	Y	D
2.7	3	74	••	•	_	•	_	-	_		•						
		873	•		882			891			900			909			918
N.C.C	000		CITC.	CAC		CTC	A C A		ACC	ראַר		ልሮጥ	GCC		GGG	DAA	
ATC	GCC	CIG	GIC	CAG	GAG	GIC	ngn	GAC	AGC	CAC							
						v	R	D		u	L	Th.	Δ.	v	G	K	L
1	A	ь	v	Q	E	V	K	ט	3	п	υ.		Α.	•	G		Ð
					226			945			954			963			972
		927			936								ma c		omo	<b>&gt;</b> CM	
CTG	GAC	AAC	CTC	TAA	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAL	TAC	GIG	GTC	AGT	GAG
L	D	N	L	N	Q	D	A	Þ	D	T	Y	Н	. ¥	V	v	S	E
		981			990			999			1008			1017			1026
CCA	CTG	GGA	CGG	AAC	AGC	ጥልጥ	N N C										
							MAG	GAG	CGC	TAC	CTG	TIC	·G1G	TAC	AGG	CCT	GAC
P																	
	L	G	 R	 N	 s			E			CTG  L			TAC  Y	AGG  R	CCT  P	GAC  D
	L	G	 R	N						Y	L		v	Υ			
		1035			 S 1044	Y	ĸ	E 1053	R	Y	L 1062	F	v :	 У 1071	R	P	D D
CAG		1035			 S 1044	Y	ĸ	E 1053	R	Y	L 1062	F	v :	 Y 1071		P	D D
	GTG	1035 TCT	GCG	GTG	S 1044 GAC	Y AGC	K	E 1053 TAC	R	Y	L 1062 GAT	F GGC	V TGC	Y 1071 GAG	R	P TGC	D 1080 GGG
	GTG	1035 TCT	GCG	GTG	S 1044 GAC	Y AGC	K	E 1053 TAC	R	Y	L 1062 GAT	F GGC	V TGC	Y 1071 GAG	R	P TGC	D 1080 GGG
	GTG	1035 TCT	GCG	GTG	S 1044 GAC	Y AGC	K	E 1053 TAC	R	Y	L 1062 GAT	F GGC	V TGC	Y 1071 GAG	R	P TGC	D 1080 GGG
	GTG  V	1035 TCT  S	GCG 	GTG  V	S 1044 GAC	Y AGC	K TAC	E 1053 TAC  Y	R TAC	Y GAT	L 1062 GAT  D	F GGC	V TGC	Y 1071 GAG  E	R CCC	P TGC	D 1080 GGG  G
Q	GTG 	1035 TCT  S	GCG 	GTG	S 1044 GAC  D	Y AGC	K TAC	E 1053 TAC  Y	R TAC	Y GAT	L 1062 GAT  D	F GGC	V TGC	Y 1071 GAG  E	R CCC	P TGC	D 1080 GGG  G
Q	GTG V GAC	1035 TCT  S 1089	GCG  A	V AAC	S 1044 GAC  D 1098	AGC	TAC	E 1053 TAC Y 1107 GCC	R TAC Y	GAT D	L 1062 GAT D D	F GGC	V TGC C	Y 1071 GAG E	R CCC P	P TGC C	D 1080 GGG  G
Q AAC	GTG V GAC	1035 TCT S 1089	GCG A	GTG V AAC	S 1044 GAC  D 1098 CGA	AGC S	TAC Y CCA	E 1053 TAC Y 1107 GCC	R TAC Y ATT	GAT D	L 1062 GAT D 1116 AGG	F GGC G	V TGC C	Y  1071 GAG E  1125 TCC	R CCC P	P TGC C TTC	D 1080 GGG  G 1134 ACA
Q AAC	GTG V GAC	1035 TCT S 1089	GCG A	GTG V AAC	S 1044 GAC  D 1098 CGA	AGC S	TAC Y CCA	E 1053 TAC Y 1107 GCC	R TAC Y ATT	GAT D	L 1062 GAT D 1116 AGG	F GGC G	V TGC C	Y  1071 GAG E  1125 TCC	R CCC P	P TGC C TTC	D 1080 GGG  G 1134 ACA
Q AAC	GTG V GAC	1035 TCT S 1089	GCG A TTC	GTG V : AAC	S 1044 GAC  D 1098 CGA	AGC S GAG	TAC	E 1053 TAC  Y 1107 GCC	R TAC Y ATT	GAT D GTC	L 1062 GAT D 1116 AGG	F GGC  G TTC	V TGC C TTC	Y 1071 GAG  E 1125 TCC	R CCC P CGG	P TGC  C TTC	D 1080 GGG  G 1134 ACA
Q AAC	GTG V GAC	1035 TCT S 1089 ACC	GCG A TTC	GTG V : AAC	S 1044 GAC D 1098 CGA R	Y AGC	TAC Y CCA	E  1053 TAC Y  1107 GCC A	R TAC Y ATT	GAT D GTC	L 1062 GAT  D 1116 AGG  R	F GGC  G TTC	V TGC C TTC	Y  1071 GAG E  1125 TCC S	R CCC P	P TGC C	D 1080 GGG G 1134 ACA T
Q AAC N	GTG V GAC	1035 TCT S 1089 ACC T	GCG A TTC	GTG V AAC N	S 1044 GAC  D 1098 CGA  R 1152 GCC	Y AGC	TAC Y CCA P	E 1053 TAC Y 1107 GCC A 1161	R TAC Y ATT	GAT D GTC V	L 1062 GAT  D 1116 AGG  R	F GGC TTC F	V TGC C TTC	Y  1071 GAG E  1125 TCC S  1179 GGG	R CCC P CGG R	P TGC  C TTC  :F	D 1080 GGG G 1134 ACA T
Q AAC N	GTG V GAC	1035 TCT S 1089 ACC T	GCG A TTC	GTG V AAC	S 1044 GAC D 1098 CGA R 1152 GCC	Y AGC	K TAC	E 1053 TAC	R TAC Y ATT I	GAT D GTC V	L 1062 GAT D 1116 AGG R 1170 GCG	F GGC GCC	V TGC C C C C C C C	Y 1071 GAG E 1125 TCC S 1179 GGG	R CCC P CGG R GAC	P TGC C TTC :F	D 1080 GGG G G ACA ACA T T 1188 GTA
Q AAC N	GTG V GAC	1035 TCT S 1089 ACC T	GCG A TTC	GTG V AAC	S 1044 GAC D 1098 CGA R 1152 GCC	Y AGC	K TAC	E 1053 TAC	R TAC Y ATT I	GAT D GTC V	L 1062 GAT D 1116 AGG R 1170 GCG	F GGC GCC	V TGC C C C C C C C	Y 1071 GAG E 1125 TCC S 1179 GGG	R CCC P CGG R	P TGC C TTC :F	D 1080 GGG G G ACA ACA T T 1188 GTA
Q AAC N GAG	GAC D GTC	1035 TCT S 1089 ACC T 1143	GCG A TTC	V AAC	S 1044 GAC D 1098 CGA R 1152 GCC A	Y AGC	K TAC Y CCA P	E 1053 TAC	R TAC Y ATT I CTG	GAT D GTC V CAT	L 1062 GAT D 1116 AGG R 1170 GCG A	F GCC A	V TGC C TTC F	Y 1071 GAG E 1125 TCC S 1179 GGG G	R CCCC P CGG R GAC	P TGC C TTC F	D 1080 GGG G G 1134 ACA T 1188 GTA CTA V
Q AAC N	GAC D GTC	1035 TCT S 1089 ACC T	GCG A TTC	V AAC	S 1044 GAC D 1098 CGA R 1152 GCC A 1206	Y AGC	K TAC Y CCA P	E 1053 TAC	R TAC Y ATT I CTG	GAT D GTC V CAT H	L 1062 GAT D 1116 AGG R 1170 GCG A	F GGC TTC F GCC A	V TGC C C P	Y 1071 GAG E 1125 TCC S 1179 GGG G	R CCC P CGG R GAC	P TGC C TTC F	D 1080 GGG G G ACA ACA T T 1188 GTA

Fig. 13(D) (Sheet 2 of 3)

ecic	GAG	ATC	GAC	GCT	CTC	ТАТ	GAC	GTC	TAC	CTG	GAT	GTC	CAA	GAG	AAA	TGG	GGC
A	E	I	D	A:	L L	Y	D	v	Y	L	. D	v	Q	E	ĸ	w	G
	,	1251		1	260			1269			1279			1207			1296
באנה														AGC			
L	E	D	, <b>v</b>	M	L	M	G	D	F	N	A	G	С	S	Y	v	R
	:	1305		:	L314		:	1323	••	:	1332		:	1341		:	1350
CCC	TCC	CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	ccc	ACC	TTC	CAG	TGG	CTG
. Ъ	s	Q	W	S	S	I	R	L	W	T	S	P	T	F	Q	W	L
	;	1359		3	L368		:	1377		3	L386			L395		]	L404
ATC	ccc	GAC												GCC			AGG
I.	P	D	s	A	D	·T	T	A	T	P	T	H	С	A	Y	D	R
													•				
														.449			
ATC	GTG	GIT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	ccc	GAC	TCG	GCT	CTT
I	v	v	Α	G	M	L L	L	R	G	A	v	v	<b>P</b>	D	s	A	L:
		1167			175			1405			100		-	L503			1512
ccc														GCC			
·																	
P	F	N	F	Q	A	A	. <b>Y</b>	G	. T	s	D	Q	L	A	Q	A	I
•		1521			1530			1539		1	548						
AGT			TAT									3 '					
s	D	н .	. Y	p	ν	Е	ν.	M	L	к	*					•	

Fig. 13(D) (Sheet 3 of 3)

#### 58/113

#### **pAS102**

```
mRNA
                                                    PRI
                                                               06-MAR-1995
LOCUS
            PAS102.DNA
                         1566 bp
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS102)
ACCESSION
NID
            DNase I.
KEYWORDS
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
MHDNASE1.dna) (See Figure 2)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic
  TITLE
fibrosis
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
  MEDLINE
            91067672
                                  440 g
                                           312 t
                         469 c
BASE COUNT
                345 a
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC
      781 GGCCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
      841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
      901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
      961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
     1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
     1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
     1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
     1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
     1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
     1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC
     1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
     1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
     1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
     1561 AAGTGA
11
```

LOCUS	FDDNASE1	)2 1566 BI	SS-DNA	S	n 23-	-MAR-2001
DEFINITION	1 -					
ACCESSION	_					•
KEYWORDS	_					
SOURCE	_					
BASE COUNT	345 1	468 C	440 G	313 T	0 OTHER	
ORIGIN	_					•
1	ATGGGATGGA	GCTGTATCAT	CCTCTTCTTG	GTAGCAACAG	CTACAGGTGT	CCACTCCCAG
61	GTGCAGCTGG	TGCAGTCTGG	GGCAGAGGTG	AAAAAGCCTG	GGGCCTCAGT	GAAGGTGTCC
121	TGCAAGGCTT	CTGGCTACAC	CTTCAGTGCC	TACTGGATAG	AGTGGGTGCG	CCAGGCTCCA
181	GGAAAGGGCC	TCGAGTGGGT	CGGAGAGATT	TTACCTGGAA	GTAATAATTC	TAGATACAAT
241	GAGAAGTTCA	AGGGCCGAGT	GACAGTCACT	AGAGACACAT	CCACAAACAC	AGCCTACATG
301	GAGCTCAGCA	GCCTGAGGTC	TGAGGACACA	GCCGTCTATT	ACTGTGCAAG	ATCCTACGAC
361	TTTGCCTGGT	TTGCTTACTG	GGGCCAAGGG	ACTCTGGTCA	CAGTCTCCTC	AGCCTCCACC
421	AAGGGCCCAT	CGGTCTTCCC	CCTGGCACCC	TCCTCCAAGA	GCACCTCTGG	GGGCACAGCG
481	GCCCTGGGCT	GCCTGGTCAA	GGACTACTTC	CCCGAACCGG	TGACGGTGTC	GTGGAACTCA
541	GGCGCCCTGA	CCAGCGGCGT	GCACACCTTC	CCGGCTGTCC	TACAGTCCTC	AGGACTCTAC
601	TCCCTCAGCA	GCGTGGTGAC	CGTGCCCTCC	AGCAGCTTGG	GCACCCAGAC	CTACATCTGC
661	AACGTGAATC	ACAAGCCCAG	CAACACCAAG	GTGGACAAGA	AAGTTGAGCC	CAAATCTTGT
721	GACAAAACTC	ACACATGCTG	TGTCGAGTGT	CCACCGTGTC	CAGCACCAGA	GGGGAGCGGC
				ACATTTGGGG		GTCCAATGCC
841	ACCCTCGTCA	GCTACATTGT	GCAGATCCTG	AGCCGCTACG	ACATCGCCCT	GGTCCAGGAG
				AAGCTGCTGG		
961				CCACTGGGAC		
1021				TCTGCGGTGG		
1081	GGCTGCGAGC	CCTGCGGGAA	CGACACCTTC	AACCGAGAGC	CAGCCATTGT	CAGGTTCTTC
1141				ATTGTTCCCC		
1201				GTCTACCTGG		
1261				AATGCGGGCT		
1321	CAGTGGTCAT	CCATCCGCCT	GTGGACAAGC	CCCACCTTCC	AGTGGCTGAT	CCCCGACAGC
1381				GCCTATGACA		
1441				GCTCTTCCCT		
1501	GGCCTGAGTG	ACCAACTGGC	CCAAGCCATC	AGTGACCACT	ATCCAGTGGA	GGTGATGCTG
1561	AAGTGA					

Fig. 14(B)

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#### pAS302

```
FDDNASE302 1575 BP SS-DNA
                                                    SYN
                                                              29-AUG-2000
LOCUS
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
FEATURES
     frag
                    10..1575
                     /note="1 to 1566 of FdDNase102correct"
BASE COUNT
                         474 C
                                 442 G
                                           313 T
ORIGIN
        1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
     241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
     301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
     481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
     541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
      661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
     721 AAATCTTGTG ACAAAACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG
     781 GGGAGCGGCG GGCTGAAGAT CGCAGCCTTC AACATCCAGA CATTTGGGGA GACCAAGATG
     841 TCCAATGCCA CCCTCGTCAG CTACATTGTG CAGATCCTGA GCCGCTACGA CATCGCCCTG
     901 GTCCAGGAGG TCAGAGACAG CCACCTGACT GCCGTGGGGA AGCTGCTGGA CAACCTCAAT
     961 CAGGACGCAC CAGACACCTA TCACTACGTG GTCAGTGAGC CACTGGGACG GAACAGCTAT
     1021 AAGGAGCGCT ACCTGTTCGT GTACAGGCCT GACCAGGTGT CTGCGGTGGA CAGCTACTAC
    1081 TACGATGATG GCTGCGAGCC CTGCGGGAAC GACACCTTCA ACCGAGAGCC AGCCATTGTC
    1141 AGGTTCTTCT CCCGGTTCAC AGAGGTCAGG GAGTTTGCCA TTGTTCCCCT GCATGCGGCC
    1201 CCGGGGGACG CAGTAGCCGA GATCGACGCT CTCTATGACG TCTACCTGGA TGTCCAAGAG
    1261 AAATGGGGCT TGGAGGACGT CATGTTGATG GGCGACTTCA ATGCGGGCTG CAGCTATGTG
     1321 AGACCCTCCC AGTGGTCATC CATCCGCCTG TGGACAAGCC CCACCTTCCA GTGGCTGATC
    1381 CCCGACAGCG CTGACACCAC AGCTACACCC ACGCACTGTG CCTATGACAG GATCGTGGTT
    1441 GCAGGGATGC TGCTCCGAGG GGCCGTTGTT CCCGACTCGG CTCTTCCCTT TAACTTCCAG
    1501 GCTGCCTATG GCCTGAGTGA CCAACTGGCC CAAGCCATCA GTGACCACTA TCCAGTGGAG
    1561 GTGATGCTGA AGTGA
//
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Fig. 14(C)

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			9			18	٠		27			36			45			54
5,	ATG	GGA		AGC	TGT		ATC										GTC	CAC
	М	G	W	S	С	I	Ι	L	F	L	V	Α	Т	A	Т	G	٧	н
			63			. 72			81			90			99		•	108
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT			GAG	GTG	A.A.A	AAG	CCT	GGG	GCC	TCA
	s	0	v	0	L	v	0	s	 G		 E	 v	к		 P			
	•	*	•	•	_	-	-		_		-	•		•	-	-		Ū
			117			126			135			144			153			162
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	AGT	GCC	TAC	TGG	ATA	GAG
	v	ĸ	v	S	С	ĸ	A	s	G	Y	T	F	s	A	Y	W	I	E
			171			180			189			198			207			22.6
	TGG	GTG		CAG	GCT	•											TTA	216 CCT
	W	V	R	Q	Α,	P	G	K	G	L	E	W	V	G	E	I	L	P
			225			234			243			252			261			270
	GGA	AGT	AAT	TAA	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT
	 G	s	N	N	s	 R	Y		E		 F			 R	v	т		 Т
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	202	CAC	279	mcc.	202	288	202	000	297	N TO C	CNO	306	100		315		mam	324
					ACA		~~~									AGG	101	GAG
	R	D	T	s	T	N	T	A	Y	M	E	L	s	s	L	R	s	E
			333			342			351			360			369		_	378
	GAC	ACA		GTC	TAT		TGT	GCA										
•																		
	D	Т	A	V	Y	Y	С	A	R	S	Y.	D	r	A	W	F	A	Y
			387			396			405			414			423			432
	TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC		TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG
	W	G	Q	G	T	L	v	T	ν		s	A ·	s	T	ĸ	G	P	s
						450			450			450			427			405
	GTC	TTC	441 CCC	CTG	GCA	450 CCC	TCC	TCC	459 AAG	AGC	ACC	468 TCT		GGC	477 ACA	GCG	GCC	486 CTG
							·									<del></del> -	, <u>-</u>	
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			495			504			513			522			531			540
	GGC			GTC	AAG			TTC								TGG	AAC	TCA
	 G	c	L L	v	к	D	 Y	F	 P	 E	 P	v	T	 v	 s	w	 N	s
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	ccc	ccc	549	200	»CC	558	CITC	242	567 ACC	Trans	ccc	576	CTC	Cm s	585	TC-C	mc ·	594
				ACC.	AGC	-:-						GCT			CAG		1CA	
	C	A	L	T		G					P	A	v	L	Q	s	s	Ģ
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Fig. 14(D) (Sheet 1 of 3)

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							V	<i>—</i> 1 .	L L	•							
		603			612			621			630			639			648
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG				TCC					ACC	CAG
•	37			_		.,	11		V	P	s	s	s	L	G	T	Q
L	Y	S	L	ş	s	V	V	T	V	P	5	3	3	b	G	•	Q
													•				
		657			666			675			684			693			702
·ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA
									·								
T	Y	I	С	N	v	N	н	к	P	s.	· N	T	K	V	D	K	K
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		711			720			729			738			747			756
													000		maa	~~	
GTT	GAG	ccc	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	TGT	GIG	GAG	TGC	CCA	CCG
v	E	P	K	S	C	a	K	T	H	T	С	С	V	£	С	P	P
																•	
		765			774			783			792			801			810
TGC	CCA	GCA	CCT	GAA	GGG	AGC	GGC	GGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG
С	P	h	P	E	G	•	C	G	٠,	ĸ	I	A	A	F	N	1	0
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		819			828			837			846			855			864
ACA	TTT	GGG	GAG	ACC	AAG	ATG	TCC	AAT	GCC	ACC	CTC		AGC	TAC	ATT	GTG	CAG
T	F	G	E	T	ĸ	М	S	N	A	T	L	V	S	Y	I	v	Q
		873			882			891			900			909			918
ATC	CTG	AGC	CGC	TAC	GAC	ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CTG
т	L	s	R	v	D	т	A	τ.	v	O	E	v	R	D	S	н	t.
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		022			936			945			954			963			972
		927															
ACT	GCC	GIG	GGG	AAG	CIG	CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT
T	A	ν	G	K	L	L	D	N	L	Ŋ	Q	D	A	Þ	D	T	Y
		•															
		981			990			999		:	1008		:	1017			1026
CAC	TAC	GTG	GTC	AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG
																	·
н	Y	ν	v	s	E	P	r.	G	R	Ν,	s	Y	K	E	R	Y	L
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		1035			1044			1053			1062			1071		•	080
mmo			200						000		GAC	NGC.			TAC		
110	GIG	INC	MGG	CCI	GAC	CAG	616	101	GCG	GIG	GAC	AGC	Inc	1110	IAC	GAI	GAI
F	V	Y	R	P	D	Q	V	5	A	V	D	5	Y	ĭ	¥	D	D
		1089									1116						
GGC	TGC	GAG	CCC	TGC	GGG	AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	TTA	GTC	AGG
·																	
G	С	E	P	. с	G	N	D	T	F	N	R	E	₽	A	I	V	R
	,	1143			1152			1161		. 1	1170			1179		3	188
<b>J</b> m√											GCC						
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F	r	5	ĸ	r	ľ	E	V	. ĸ	Ē	r	ų	•	٧	r	ט	п	W.
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	:	1197		:							1224			1233		1	242
						T7 (	•	7	1 1		١.						

Fig. 14(D) (Sheet 2 of 3)

GCC	CCG	GGG	GAC	GCA	GTA.	GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	
A	P	G	D	Α	v	A	E	I	.D	A	L	Y	D	v <sub>.</sub>	Y	L	D	
								1260			1278						1200	
		1251															1296	
GTC	CAA	GAG	AAA	TGG	GGC	TIG	GAG	GAC	GIC	ATG	TŢĢ	ATG	GGC	GAC	TTC	AA'I'	GCG	
v		E	ĸ	 W	G	T.	Е	D	v	м	L	м	G	D	F	NI		
•	Q	-	**	••	٠	~	_	_	•	••	_	••	Ü		-	••	•••	
	;	1305		:	1314		:	1323		•	1332	:	;	1341			1350	•
GGC	TGC	AGC	TAT	GTG	AGA	ĊCC	TCC	CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	•
G	С	S	Y	v	R	P	s	Q	W	S	S	I	R	${f r}$	W	T	s	
				_									_					
		1359									1386						1404	
CCC	ACC	TTC	CAG	TGG	CTG	ATC	CCC	GAC	AGC	GCT	GAC	ACC	ACA	GCT	ACA	CCC	ACG	
	~~~	F									D	т	т			 P		
P	T	F	Q	w	п	1	r	ט	3	^	D	•	1	A	1	F	1	
	:	1413		:	1422		3	L <b>4</b> 31		:	1440		:	L449			1458	
CAC	TGT	GCC	TAT	GAC	AGG	ATC	GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	
H	С	A	Y	D	R	I	v	v	A	G	M	L	L	R	G	A	v	
		1467									1494						1512	
GTT	CCC	GAC	TCG	GCT	CTT.	ccc	TTT	AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	
		ם י	s		L	P		NJ		0	 A	Δ.		G	L	s		
v	r		3		~	•			٠	v			•	G		,	b	
		1521			1530			.539		:	1548			L557			1566	
CAA																	TGA	3,
Q	L	A	Q	A	I	s	D	н	. <b>Y</b>	P	v	E	v	M	L	ĸ	*	

Fig. 14(D) (Sheet 3 of 3)

#### 64/113

#### pAS103

mRNA

1560 bp

PAS103.DNA

LOCUS

//

06-MAR-1995

PRI

```
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS103)
ACCESSION
NID
KEYWORDS
            DNase I.
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
MHDNASE1.dna)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic
  TITLE
fibrosis
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
            91067672
  MEDLINE
                                  436 g
                                           312 t
                344 a
                         468 c
BASE COUNT
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGGCTG
      781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
      841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
      901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
      961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
     1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
     1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
     1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
     1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
     1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
     1441 CGAGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
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SOURCE
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     fraq
                     /note="1 to 78 of 102linker [Split]"
BASE COUNT
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                         467 C
                                  436 G
                                           313 T
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       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
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      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
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      721 GACAAAACTC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGCGGGCTG
      781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
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Fig. 15(B)

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ACCESSION
KEYWORDS
SOURCE
                     Location/Qualifiers
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                     /note="1 to 72 of 103/107linker"
     frag
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                     /note="1 to 78 of 102linker [Split]"
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                                         313 T
                        473 C
BASE COUNT
                345 A
ORIGIN
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       61 CACTCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
      181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
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      781 GGCGGGCTGA AGATCGCAGC CTTCAACATC CAGACATTTG GGGAGACCAA GATGTCCAAT
      841 GCCACCCTCG TCAGCTACAT TGTGCAGATC CTGAGCCGCT ACGACATCGC CCTGGTCCAG
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      961 GCACCAGACA CCTATCACTA CGTGGTCAGT GAGCCACTGG GACGGAACAG CTATAAGGAG
     1021 CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT
     1081 GATGGCTGCG AGCCCTGCGG GAACGACACC TTCAACCGAG AGCCAGCCAT TGTCAGGTTC
     1141 TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTC CCCTGCATGC GGCCCCGGGG
     1201 GACGCAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG
     1261 GGCTTGGAGG ACGTCATGTT GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC
     1321 TCCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC
     1381 AGCGCTGACA CCACAGCTAC ACCCACGCAC TGTGCCTATG ACAGGATCGT GGTTGCAGGG
     1441 ATGCTGCTCC GAGGGGCCGT TGTTCCCGAC TCGGCTCTTC CCTTTAACTT CCAGGCTGCC
     1501 TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG
     1561 CTGAAGTGA
11
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Fig. 15(C)

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	ÞΨC	433	9 TCC	460	ጥርጥ	18 2TA					ста				45 ACA		CTC	
,																		
	Ħ	G	W.	S	С	I	I	L	F	L	v	A	T	A	T	G	V	Н
			63			72			81			90			99			108
	TCC	CAG		CAG	CTG		CAG	TCT			GAG		AAA	AAG	CCT			
	s	Q	ν	Q	L	V	Q	S	G	A	E	V	K	K	₽	G	A	S
			117			126		•	135			144			153	•		162
	GTG	AAG		TCC	TGC			TCT		TAC	ACC			GCC.	TAC			
	v	K	v	S	C	K	A	S.	G	Y	T	F	S	A	Y	W	I	E
			171		•	180			189			198			207			216
	TGG	GTG		CAG	GCT			AAG						GGA	GAG			
	W	V	R	Q	A	P	G	ĸ	G	L	E	W	V	G	E	Ι	L	₽
			225			234			243			252			261			270
	GGA	AGT	TAA	TAA	TCT	AGA			GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	
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	G	s	IN	14	٥	Į.	•	N	5	V	r	K	G	Α.	٧	•	V	•
			279			288			297			306			315			324
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
	R	D	T	s	T	N	T	A	Y	м	E	L	s	s	L	R	s	E
	~~~		333	~~~	m> m	342									369			
	GAC	ACA			1A1	IAC	161			100	TAC	GAC			TGG			
	D	T	A	v	Y	Y	С	A	R	s	Y	D	F	A	W	F	A	Y
									405			43.4			407			422
	TGG	ccc	387		<b>አ</b> ርጥ	396 Стс		ACA	405 GTC		TCA	414 GCC		ACC	423 AAG		CCA	432 TCG
	W	G	Q	G	T	L	V	T	V	S	S	A	S	T	K	G	P	S
			441			450			459			468			477			486
	GTC				GCA			TCC			ACC			GGC	ACA	GCG	GCC	_
								<u></u>								·		
	v	F	P	L	A	₽	S	S	K	S	T	S	G	G	T	A	A	L
			495			504			513	•		522			531			540
	GGC	TGC	CTG	GTC	AAG	GAC								GTG	TCG	TGG	AAC	TCA
	G	С	L	ν	K	D	Y	F	P	E	Р	ν	Т	V	S	₩	N	S
			549			558			567			576			585			594
	GGC	GCC	CTG	ACC	AGC	GGC	GTG	CAC	ACC	TTC	CCG	GCT	GTC	СТА	CAG	TCC	TCA	GGA
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Fig. 15(D) (Sheet 1 of 3)

639 648 630 612 CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG L Y S L S S V V T V P S S S L G T Q 675 <sup>684</sup> 693 666 ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---T Y I C N V N H K P S N T K V D K K 711 720 729 738 GTT GAG CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC TGT GTG GAG TGC CCA CCG V E P K S C D K T H T C C V E C P P 801 774 783 792 TGC CCA GCA CCT GAA GGC GGG CTG AAG ATC GCA GCC TTC AAC ATC CAG ACA TTT \_\_\_ \_\_\_ \_\_\_ C P A P E G G L K I A A F N I Q T F 855 864 819 828 837 846 GGG GAG ACC AAG ATG TCC AAT GCC ACC CTC GTC AGC TAC ATT GTG CAG ATC CTG GETKMSNATLVSYIVQIL 882 900 891 909 873 AGC CGC TAC GAC ATC GCC CTG GTC CAG GAG GTC AGA GAC AGC CAC CTG ACT GCC S R Y D I A L V Q E V R D S H L T A 936 945 954 <del>9</del>-63 GTG GGG AAG CTG CTG GAC AAC CTC AAT CAG GAC GCA CCA GAC ACC TAT CAC TAC V G K L L D N L N Q D A P D T Y H Y 990 999 1008 1017 981 GTG GTC AGT GAG CCA CTG GGA CGG AAC AGC TAT AAG GAG CGC TAC CTG TTC GTG 1044 1053 1062 1071 TAC AGG CCT GAC GAG GTG TCT GCG GTG.GAC AGC TAC TAC TAC GAT GAT GGC TGC --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---Y R P D Q V S A V D S Y Y Y D D G C 1125 . 1089 1098 1107 1116 GAG CCC TGC GGG AAC GAC ACC TTC AAC CGA GAG CCA GCC ATT GTC AGG TTC TTC --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---EPCGNDTFNREPAIVRFF 1170 1152 1161 1179 TCC CGG TTC ACA GAG GTC AGG GAG TTT GCC ATT GTT CCC CTG CAT GCG GCC CCG S R F · T E V R E F A I V P L H A A P 1206 1215 1224 1233 1242 1197

Fig. 15(D) (Sheet 2 of 3)

GGG	GAC	GCA	GTA	GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA
G	D	A	ν	A	E	1	D	A	L	Y	D	V	Y	L	Đ	v	Q
		L251												1287			
GAG	AAA	TGG	GGC	TTG	GAG	GAC	GTC	ATG	TTG	ATG	GGC	GAC	TTC	TAA	GCG	GGC	TGC
Ε	ĸ	W	G	L	E	D	v	M	L	M	G	D	F	N	A	G	С
		1305			1314		:	1323			1332		:	1341		:	1350
AGC	TAT	GTG												ACA	AGC	ccc	ACC
S	Y	v	R	₽.	S	0	W	s	s	I	R	L	W	T	s	₽	T
-	-	-				-											
		1359			1368		:	1377		:	1386		:	1395		:	1404
date.														CCC			
									,-								
F	0	W	L	т	ъ.	· D	s	A	D	T	T	A	т	P	т	H	æ
•	~	**	-	-	•	_	_										
		1413			1422			1431			1440		:	1449			1458
CCC														GCC			
	v	n	R	_	17	37	A	G	м	L	L	R	G	A	v	v	P
Α.	-	ט	K	-	٠	•	••	•		_	_						
		1467			1476			1485			1494		:	1503			1512
														AGT			CTG
GAC																	
		7	L	Ð	F	N	F	0	Α	A	Y	G	L	s	D	0	L
U	3	~	u	E	-	.,	٠	×	••	••	•	•	_	_	_	•	_
		1521			1530			1539			1548			1557			
000														AAG	TGA	3 '	
حال	CAA	الان	AIC	AG1			****									-	
	0	Α.	I	s	D	н	Y	P	v	E	ν	м	L	к			

Fig. 15(D) (Sheet 3 of 3)

### 70/113

#### pAS104

```
PRI
                                                              06-MAR-1995
                                    mRNA
            PAS104.DNA
                         1560 bp
DEFINITION Humanised HMFG1 Fab'2 fused to human DNase I (pAS104)
Position 924 G to A by ggg to gag
Linker GR instead of GG (position 777)
ACCESSION
NID
KEYWORDS
            DNase I.
            DNase I sequence is from assembled oligos (thus modified c/f
SOURCE
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  ORGANISM Homo sapiens
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            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  AUTHORS
            Recombinant human DNase I reduces the viscosity of cystic
  TITLE
fibrosis
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
  MEDLINE
            91067672
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11

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      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
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     1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
     1441 CGAGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
     1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGTGA
11
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			9			18												54
5′	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	м	- G	W	s	c	 I	I	L	F	L	v	A	T	A	T	G	v	н
	••	Ĭ	•		-													
			63			72			81			90			99			108
	TCC	CAG	GTG	CAG	CTG	GTG		TCT				GIG		.AAG	CCT	GGG	GCC	TCA
	s	0	v	0	L					A				ĸ	P	G	A	s
	_	•					-											
			117			126			135		٠	144			153	maa		162
	GTG	AAG	GTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC		AGT		TAC	166	ATA	GAG
	v	К	v	S	С	ĸ	A	s	G	Y	T	F	s	A	Y	· W	I	E
	<b></b>	~~~	171		~~m	180	CCX	330	189		GVG	198		CCA	207 GAG		עינאני	216
	166	GIG			GC1			AAG										
	W	v	R	Q	A	P	G	K	G	L	E	W	v	G	E	İ	L	P
			225			234			243			252			261			270
	GGA	AGT	225 AAT		TCT			TAA			TTC		GGC	CGA	GTG	ACA	GTC	
	G.	S	N	N	S	R	Y	N	E	K	F	K	G	R	V	T	V	T
			279			288			297			306			315			324
	AGA	GAC			ACA			GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
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	R.	D	T	S	T	N	T	А	ĭ	M	E	ם	3	3	b	Λ.	3	5
			333		•	342			351			360			369			378
	GAC	ACA	GCC	GTC	TAT	TAC	TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
1	 D	т			Y	Y	С.	Α	R	s	Y	D	F	A	W	F		Y
}	-	•																
			387			396			405		mc»			200	423	~~~	<b>~~</b>	432
	TGG	GGC	CAA	GGG	ACT	CTG	GIC	ACA	GIC	100				MCC	AAG			
	W.	G	Q	· · · · · · · · · · · · · ·	T	Ŀ	· <b>v</b> ·	T	v	·s·	s	A	s	T	-K-	G	P	· · · s · · ·
									450			460			477			400
	CTC	, 444.	441		GCA	450 CCC		TCC	459 AAG		ACC			GGC		GCG		486 CTG
	v	F	P	L	A	P	S	S	K	S	T	S	G	G	T	A	A	L
		•	495	i		504			513			522			531		•	540
	GGC	TGC			AAG			TTC						GTG	TCG	TGG	AAC	TCA
	G	С	L	٧	ĸ	D	Y	F	₽	Ē	₽	V	Т	V	s	W	N	S
			549			558									585			5'94
•															CAG			
															 Q			
	G	Α	L	T	3	G	v	ŗ.	1	r	Ľ	^	٠	ם	×	٠		•

Fig. 16(C) (Sheet 1 of 3)

							1	"	1	<b>,</b>							
		603			612			621			630			639			648
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG
L	Y	·s	L	s	s	v	v	T	ν	P	s	s	s	L	G	т	Q
_	•	•	_	_	-			-		-	-	-	-	_	_	-	~
		657			666			675			684			693			702
100	<b>71.0</b>		mcc			2.20	CNC		ccc	»cic					GAC		
ACC	IAC	AIC	160	AAC	GIG	WAI	CAC	AAG	CCC.	AGC	ممر	٠	AAG	GIG	GAC	AAG	P.A.A
T	Y	I	С	N	V	N	н.	K	P	S	N	Т	K	v	D	K	K
		711			720			729						747			756
GTT	GAG	CCC	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	TGT	GTG	GAG	TGC	CCA	CCG
v	E	P	K	S	С	D	K	T	H	T	C	С	ν.	E	С	P	P
		765			774			783			792			801			810
TGC	CCA	GCA	CCT	GAA	GGC	AGG	CTG	AAG	ATC	GCA	GCC	TTC	AAC	ATC	CAG	ACA	TTT
С	₽	A	P	E	G	R	L	ĸ	I	A	A	F	N	I	Q	T	F
													•		_		
		819			828			837			846			855			864
GGG	GAG	ACC	AAG	ATG	TCC	ААТ	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	
G	E	T	ĸ	M	s	N	A	T	L	v	s	Y	r	v	0 -	I	L
G		•		23	3	14	•	•	_	•	_	-	-	٠	Q	_	ט
		873			882			891			900			909			918
		_	~~~			~~~	~~~		~~	~~~		CNC	200		ama		
AGC	CGC	TAC	GAC	ATC	GCC	CIG	GIC	CAG	GAG	GIC	AGA	GAC	AGC	CAC	CTG	ACT	GCC
5	R	Y	ע	1	A	ь	V	Ų	£	V	R	ט	s	H	L	T	A
		022			026			045			054			0.63			
		927			936			945			954			963			972
GTG	GAG	AAG	CTG	CTG	GAC	AAC	CIC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC
,V	Ξ	K	L	L	D	N	L	N	Q	D	A	P	D	T	Y	H	Y
										_		•					
		981			990			999			1008			1017			1026
GTG	GTC	AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC			GAG	CGC	TAC	CTG	TTC	GTG
									-								
V	v	s	E	P	L	G	R	N		Y	K	E	R	Y	L	F	V
												-		-			
	:	1035			1044			1053		3	1062		1	1071		1	080
TAC	AGG	CCT	GAC						GAC	AGC	TAC	TAC	TAC	GAT	GAT	GGC	TGC
Y	R	₽	D	Q	v	S	A	v	D	S	Y	Y	Y	D	D	G	С
	:	1089		:	1098		:	1107		1	1116		1	L125		1	134
GAG	CCC	TGC	GGG	AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	TTA	GTC	AGĢ	TTC	TTC
E	P	С	G	N	D	T	F	N	R	E	₽	A	I	v	R	F	F
	:	1143			1152	•		1161		1	L170		1	179		1	188
TCC															GCG	-	
															Α		
-		•	•	_	•	•	-	-	.,	-	•	-	-	••	••	••	•
		1107			1205			1215		,	224		,	.233			242
		1131			1200			- 273	_		. 444		,	ددے		1	242

Fig. 16(C) (Sheet 2 of 3)

GGG	GAC	GCA	GTA	GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA
G	D	A	v	Α	E	ı	D	A	L	Y	D	v	Y	L	D	v	Q
	1	1251		1	L260		1	1269		:	1278		:	1287		:	1296
GAG														AAT			
		~ Tai		 T.					 T.			ם	F	N	 A		
Ü		**		-		•	•	••	_		Ū	_	•		••	Ū	Ū
		1305		:	1314		;	1323			1332		:	1341			1350
AGC	TAT	GTG	AGA	CCC	TCC	CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	CCC	ACC
s	Y	v	R.	₽	S	Q	W	S	S.	Ι	R	L	W	T	S	P	T
	:	1359			1368		:	1377			1386		;	1395		:	1404
TTC	CAG	TGG	CTG	ATC	ccc	GAC	AGC	GCT	GAC	ACC	ACA	GCT	ACA	CCC	ACG	CAC	TGT
															<u></u>		
F	Q	W	L	I	P	D	s	A	D	T	T	A	T	P	T	H	С
		1413			1 422			1/21			1440			1449			1/50
CCC														GCC			
	TAI		AGG														
A	Y	D	R	ľ	V	v	A	G	M	L	L	R	G	A	v	v	₽.
		1467		1	1476			1485			1494		:	1503			1512
GAC	TCG	GCT	CTT	ccc	TTT	AAC	TTC	CAG	GCT	GCC	TAT	GGC	CTG	AGT	GAC	CAA	CTG
Đ	S	A	L	P	F	N	F	Q	A.	A	Y	G	ь	S	D	Q	L
		1521			1530		:	1539		:	1548		:	L557			
GCC	CAA	GCC	ATC	AGT	GAC									AAG	TGA	3′	
												<del></del> .	=-				
A.	Q	A	I	S	D	H	Y	P	V	E	v	M	Ľ	K	*		

Fig. 16(C) (Sheet 3 of 3)

#### pAS105

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LOCUS
            PAS105.DNA
                         1578 bp
                                    mRNA
                                                     PRI
                                                               06-MAR-1995
            Humanised HMFG1 Fab'2 fused to human DNase I with SV40
DEFINITION
NLS (pAS105)
ACCESSION
NID
KEYWORDS
            DNase I.
SOURCE
            DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
 ORGANISM
           Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  TITLE
            Recombinant human DNase I reduces the viscosity of cystic
fibrosis
            sputum
  JOURNAL
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
 MEDLINE
            91067672
BASE COUNT
                353 a
                         473 c
                                  442 g
                                           310 t
ORIGIN
       1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAG GCGGGCTGAA GATCGCAGCC
      781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
      841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
      901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
      961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
    1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
    1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
    1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
    1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
    1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
```

1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA

→ NLS

1561 AAGAAGCGCA AGGTTTGA

11

#### 76/113

```
LOCUS
            FDDNASE105 1578 BP SS-DNA
                                                    SYN
                                                              25-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
FEATURES
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     frag
                     /note="1 to 1578 of PAS105.dna [Split]"
                     721..780
     frag
                     /note="1 to 60 of 101/105linker"
     frag
                     join (721..>735, <736..>759, <760..>780)
                     /note="1 to 80 of 102linker [Split]"
                353 A
                                           311 T
BASE COUNT
                         471 C
                                  443 G
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGTCC ACCGTGTCCA GCACCAGAGG GCGGGCTGAA GATCGCAGCC
      781 TTCAACATCC AGACATTTGG GGAGACCAAG ATGTCCAATG CCACCCTCGT CAGCTACATT
      841 GTGCAGATCC TGAGCCGCTA CGACATCGCC CTGGTCCAGG AGGTCAGAGA CAGCCACCTG
      901 ACTGCCGTGG GGAAGCTGCT GGACAACCTC AATCAGGACG CACCAGACAC CTATCACTAC
      961 GTGGTCAGTG AGCCACTGGG ACGGAACAGC TATAAGGAGC GCTACCTGTT CGTGTACAGG
    1021 CCTGACCAGG TGTCTGCGGT GGACAGCTAC TACTACGATG ATGGCTGCGA GCCCTGCGGG
    1081 AACGACACCT TCAACCGAGA GCCAGCCATT GTCAGGTTCT TCTCCCGGTT CACAGAGGTC
    1141 AGGGAGTTTG CCATTGTTCC CCTGCATGCG GCCCCGGGGG ACGCAGTAGC CGAGATCGAC
     1201 GCTCTCTATG ACGTCTACCT GGATGTCCAA GAGAAATGGG GCTTGGAGGA CGTCATGTTG
    1261 ATGGGCGACT TCAATGCGGG CTGCAGCTAT GTGAGACCCT CCCAGTGGTC ATCCATCCGC
    1321 CTGTGGACAA GCCCCACCTT CCAGTGGCTG ATCCCCGACA GCGCTGACAC CACAGCTACA
    1381 CCCACGCACT GTGCCTATGA CAGGATCGTG GTTGCAGGGA TGCTGCTCCG AGGGGCCGTT
    1441 GTTCCCGACT CGGCTCTTCC CTTTAACTTC CAGGCTGCCT ATGGCCTGAG TGACCAACTG
    1501 GCCCAAGCCA TCAGTGACCA CTATCCAGTG GAGGTGATGC TGAAGGGGGG CGGACCCAAA
    1561 AAGAAGCGCA AGGTTTGA
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Fig. 17(B)

11

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LOCUS
            FDDNASE105 1587 BP SS-DNA
                                                    SYN
                                                              29-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
FEATURES
                     Location/Qualifiers
     frag
                     10..1587
                     /note="1 to 1578 of FdDNase105correct"
                     join(10..>729,<790..1587)
     frag
                     /note="1 to 1578 of PAS105.dna [Split]"
     frag
                     730..789
                     /note="1 to 60 of 101/105linker"
                     join(730..>744,<745..>768,<769..>789)
     frag
                     /note="1 to 80 of 102linker [Split]"
BASE COUNT
                354 A 477 C
                                 445 G 311 T
                                                      0 OTHER
ORIGIN
        1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
      181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
      301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
      361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
      481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
      541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
      661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
      721 AAATCTTGTG ACAAAACTCA CACATGTCCA CCGTGTCCAG CACCAGAGGG CGGGCTGAAG
      781 ATCGCAGCCT TCAACATCCA GACATTTGGG GAGACCAAGA TGTCCAATGC CACCCTCGTC
      841 AGCTACATTG TGCAGATCCT GAGCCGCTAC GACATCGCCC TGGTCCAGGA GGTCAGAGAC
      901 AGCCACCTGA CTGCCGTGGG GAAGCTGCTG GACAACCTCA ATCAGGACGC ACCAGACACC
      961 TATCACTACG TGGTCAGTGA GCCACTGGGA CGGAACAGCT ATAAGGAGCG CTACCTGTTC
    1021 GTGTACAGGC CTGACCAGGT GTCTGCGGTG GACAGCTACT ACTACGATGA TGGCTGCGAG
     1081 CCCTGCGGGA ACGACACCTT CAACCGAGAG CCAGCCATTG TCAGGTTCTT CTCCCGGTTC
     1141 ACAGAGGTCA GGGAGTTTGC CATTGTTCCC CTGCATGCGG CCCCGGGGGA CGCAGTAGCC
    1201 GAGATCGACG CTCTCTATGA CGTCTACCTG GATGTCCAAG AGAAATGGGG CTTGGAGGAC
    1261 GTCATGTTGA TGGGCGACTT CAATGCGGGC TGCAGCTATG TGAGACCCTC CCAGTGGTCA
    1321 TCCATCCGCC TGTGGACAAG CCCCACCTTC CAGTGGCTGA TCCCCGACAG CGCTGACACC
    1381 ACAGCTACAC CCACGCACTG TGCCTATGAC AGGATCGTGG TTGCAGGGAT GCTGCTCCGA
    1441 GGGGCCGTTG TTCCCGACTC GGCTCTTCCC TTTAACTTCC AGGCTGCCTA TGGCCTGAGT
    1501 GACCAACTGG CCCAAGCCAT CAGTGACCAC TATCCAGTGG AGGTGATGCT GAAGGGGGGC
    1561 GGACCCAAAA AGAAGCGCAA GGTTTGA
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			9			18						36						54	
5′	ATG	GGA	TGG	AGC	TGT	ATC	ATC	CTC	TTC	TTG	GTA	GCA	ACA	GĊT	ACA	GGT	GTC	CAC	
	м М	G	w	s	с	ī	ī	L	F	L	v	Α	T	A	T	G	v	н	
,	•		63			72			81			90			99			108	
	TCC	CAG	GTG	CAG	CTG	GTG	CAG	TCT			GAG	GTG	AAA	AAG	CCT	GGG	GCC	TCA	
								÷			E	v	к		P	 G	 A	 s	
	S	Q	V 117	Q	ט	V 126	Q	3	135		L	144			153	•		162	
	GTG	AAG		TCC	TGC		GCT	TCT			ACC			GCC	TAC	TGG	ATA	GAG	
	V	ĸ	V	s	С	K	A	s	. G		T	F		Α.	Y	W	I	E	
	<b>***</b>	Omc.	171	CAG	CCM	180	CCA	እአሮ	189		C)C	198		CCA	207 GAG		тта	216 CCT	
	166	GIG	CGC	CAG	GCT		GGA	AAG											
	W	v	R	Q	A	P	G	ĸ	٠G	L	E	W	v	G	E	I	L	P	
			225			234			243			252			261			270	
	GGA	AGT	TAA	TAA	TCT	AGA	TAC	AAT	GAG	AAG	TTC	AAG	GGC	CGA	GTG	ACA	GTC	ACT	
	<b>-</b>	s	N	N	s	R	Y	N	E	K	F	K	G	R	v	T	v	T	
	Ü	Ū	•		_	••	-	-	_									•	
			279			288									315			324	
	AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG	
	R	Đ	T	s	т	N	<b>T</b>	A	Y	М	E	L	s	s	L	R ·	S	E	
			333			342			351			360			369			378	
	GAC	ACA	GCC	GTC	TAT		TGT	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC	
	D	T	A .	v	Y	Y	С	A	R	s	Y	D	F	A	W	F	A	Y .	
			387			396			405			414			423		•	432	
	TGG	GGC	CAA	GGG	ACT	CTG	GŢĊ	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	GGC	CCA	TCG	
				 G											~~~		<b>-</b>	e	
	W	G	ب	G	1	u	v	•		-	,		_	•	••	Ŭ	-	J	
			441			450			459			468			477			486	
	GIC	TTC	CCC	CTG	GCA	ccc	TCC										GCC	CTG	
	v	F	P	L	A	P	s					•							
				,														540	
	GGC	TGC	CTC	GTC	AAG	GAC	TAC	TTC	CCC	GAA	CCG	GTG	ACG	GTG	TCG	TGG	AAC	TCA	
	G	С	L	v	к	D	Y	F	P	E	P	v	T	ý	s	W	N	s	
			549	)		558			567			576			585			594	
	GGC	GCC		ACC	AGC						CCG	GCT							
			·												·				
	G	A	L	T	\$	G	V	н	Т	F	P	λ	V	L	. Q	S	S	G	

Fig. 17(D) (Sheet 1 of 3)

/9/113 603 612 621 630 639 648																		
		603			612						630			639			648	
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GIG	CCC	TCC	AGC	AGC	TTG	GGC	ACC	CAG	
						 v	v	т	v	 P	 s	s	s	L		T	Q	
L	Y	\$	L	S	S	٧		1	V	P	3	3	3	ņ	G	•	Q	
		657			666			675			684			693			702	
ACC	TAC.		TGC	AAC	GTG	AAT	CAC	AAG	ccc	AGC	AAC	ACC	AAG	GTG	GAC	AAG	AAA	
T	Y	1	C	N	v	N	Н	K	P	S	N	Т	K	V	D	K	К	
		711			720			729			738			747			756	
Cala	GAG		AAA	тст		GAC	AAA			ACA		CCA	CCG		CCA	GCA		
v	E	P	K	s	С	D,	ĸ	T	H	T	С	P	P	С	P	A	₽	
	•				~~ 4						792			801			810	
C		765	CIT/C	220	774		CCC	783		ATC			ተተተ		GAG	ACC		
E	G	G	L	ĸ	ı	A	A	F	N	I	Q	T	F	G	E	T	ĸ	
									•									
		819			828		200	837	א נדינדו	CITC	846		CALC	855 ACC	CGC	መስር	864	
ATG	100	AAT	GCC	ACC	CIC	G1C	AGC							AGC				
M	s	N	A	T	L.	v	s	Y	I	v	Q	ľ	L	s	R	Y	D	
		873			882			891		0.0	900	» cm	~~~	909	000		918	
ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA	GAC	AGC	CAC	CIG	ACT			GGG	AAG	CIG	
1	A	L	v	0	E	v	Ŕ	D	s	Н	L	T	A	v	G	ĸ	L	
				_														
		927			936			945			954	~~~	<b></b>	963		. ~	<del>9</del> 72	
CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT	CAC	TAC	GIG	GTC	AGT	GAG	
L	D	N	L	N	Q	D	A	P	D	T	Y	н	Y	v	v	s	E	
		981			990						1008						1626	
CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG	TTC	GTG	TAC	AGG	cer	GAC	
 P	D.	 G	R	N	s	Y	ĸ	E	R	Y	L	F	v	Y	R	P	D	
٠	_	٠.		-	_													
		1035			1044			1053			1062			1071			1080	
CAG	GTG	TCT	, ece		-GAC						GAT		TGC	GAG	.ccc	TGC	GGG	
	v	<u></u>	Α.										С	. E	P	С	G	
V	•	٠	•	•	_	-	-	_	-		_	-						
		1089	١.	. •	1098			1107			1116							
AAC	GAC	ACC	TTC										TTC	TCC	CGG	TTC	ACA	
		·											F	s	R	F	T	
N	ט	•	•	.,		-	-	•	•	·	••	-	_	_		•	-	
		1143	1		1152			1161			1170						1188	
GAG	GTC	AGG	GAG	TTT	, ecc	TTA	GTT	ccc	CTG	CAT	GCG	GCC	CCG		GAC	GCA	GTA	
													 D		Đ		v	
E	V	К	E	r													•	
		1197	,		1206	;		1215			1224			1233			1242	
						$\boldsymbol{F}$	ia	1	7		) )							

Fig. 17(D) (Sheet 2 of 3)

GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA	GAG	AAA	TGG	GGC
		 T	 D		 L	 Y	 D		 Y	. Г	D	v	Q	E	ĸ	w	G
	_	•		••	_	_	_	•	-				-	•			
		1251			L260		1							.287			1296
TTG	GAG	GAC	GTC	ATG	TTG	ATG	GGC	GAC	TTC	AAT	GCG	GGC	TGC	AGC	TAT	GTG	AGA
L	E	D	V	M	L	M	G	D	F	N	A	G	С	S	Y	V	R
		<b>.</b>			1314				•					1341			1350
		1305	maa		TCC												
CCC	TCC	CAG	TGG	TCA	TCC	ATC	CGC		166								
			w	S	s	т	R	L	₩	т	s	ŕ	T	F	· Q	W	L
•	5	. 2	••	-	_	-		_									
		1359		:	1368		:	1377		3	1386		:	1395		;	1404
ATC	CCC	GAC	AGC	GCT	GAC	ACC	ACA	GCT	ACA	CCC	ACG	CAC	TGT	GCC	TAT	GAC	AGG
			<b>-</b>														
I	P	D.	S	A	D	T	T	A	T	P	T	H	С	A	Y	D	R
				-							1440			1440			1.450
1.00		1413		CCC													
ATC					ATG									GAC			
	GTG	GTT	GCA	GGG 	ATG	CTG	CTC	CGA	GGG	GCC	GTT 	GTT 	CCC	GAC	TCG	GCT	CTT
	GTG	GTT	GCA  A	GGG  G	ATG  M	CTG  L	CTC  L	CGA  R	GGG  G	GCC A	GTT V	GTT  V	CCC P	GAC  D	TCG  S	GCT A	CTT L
ī	gtg  V	GTT  V 1467	GCA  A	GGG G	ATG  M 1476	CTG  L	CTC  L	CGA  R 1485	GGG  G	GCC A	GTT  V 1494	V 	CCC P	GAC  D 1503	TCG  S	GCT A	L 1512
ī	gtg  V	GTT  V 1467	GCA  A	GGG G	ATG  M	CTG  L	CTC  L	CGA  R 1485	GGG  G	GCC A	GTT  V 1494	V 	CCC P	GAC  D 1503	TCG  S	GCT A	L 1512
I	GTG V	GTT V 1467 AAC	GCA A TTC	GGG G CAG	ATG M 1476 GCT	CTG L GCC	CTC L TAT	CGA R R 1485 GGC	GGG G CTG	GCC A A AGT	GTT V 1494 GAC	CAA	CCC P CTG	GAC D 1503 GCC	TCG S S	A GCC	L 1512 ATC
I	GTG V	GTT V 1467 AAC	GCA A TTC	GGG G CAG	ATG  M 1476	CTG L GCC	CTC L TAT	CGA R R 1485 GGC	GGG G CTG	GCC A A AGT	GTT V 1494 GAC	CAA	CCC P CTG	GAC D 1503 GCC	TCG S S	A GCC	L 1512 ATC
I	GTG V TTT	GTT V 1467 AAC —— N	GCA A TTC	GGG G G CAG	ATG  M  1476  GCT  A	CTG L GCC A	CTC L TAT	CGA R 1485 GGC GGC	GGG G CTG L	GCC A A AGT	GTT  V 1494 GAC  D	CAA	CCC P CTG L	GAC D 1503 GCC A	TCG S CAA Q	A GCC	L 1512 ATC
I CCC	GTG V TTT	GTT V 1467 AAC N 1521	GCA A TTC	GGG G CAG	ATG M 1476 GCT A	CTG L GCC A	CTC L TAT Y	CGA  R 1485 GGC  G	GGG G CTG L	GCC A A AGT	GTT V 1494 GAC D	CAA	CCC P CTG	GAC D 1503 GCC A 1557	TCG S CAA Q	GCT A GCC A	L 1512 ATC  I
I CCC	GTG V TTT	GTT V 1467 AAC N 1521	GCA A TTC	GGG G CAG	ATG  M  1476  GCT  A	CTG L GCC A	CTC L TAT Y	CGA  R 1485 GGC  G	GGG G CTG L	GCC A A AGT	GTT V 1494 GAC D	CAA	CCC P CTG	GAC D 1503 GCC A 1557	TCG S CAA Q	GCT A GCC A	L 1512 ATC  I
I CCC	GTG V TTT F	GTT V 1467 AAC N 1521 CAC	GCA A TTC F	GGG G CAG Q CCA	ATG M 1476 GCT A	CTG L GCC A GAG	TAT Y GTG	CGA R 1485 GGC  G 1539 ATG	GGG G CTG L CTG	AGT S	GTT V 1494 GAC D 1548 GGG	CAA Q GGC	CCC P CTG L GGA	GAC D 1503 GCC A 1557 CCC	TCG S CAA Q Q	GCT A GCC A AAG	L 1512 ATC  I 1566 AAG
I CCC	GTG V TTT F	GTT V 1467 AAC N 1521 CAC	GCA A TTC F	GGG G CAG Q CCA	ATG M 1476 GCT A 1530 GTG	CTG L GCC A GAG	TAT Y GTG	CGA R 1485 GGC  G 1539 ATG	GGG G CTG L CTG	AGT S	GTT V 1494 GAC D 1548 GGG	CAA Q GGC	CCC P CTG L GGA	GAC D 1503 GCC A 1557 CCC	TCG S CAA Q Q	GCT A GCC A AAG	L 1512 ATC  I 1566 AAG
I CCC	GTG V TTT F	GTT V 1467 AAC N 1521 CAC H 1575	GCA A TTC F TAT	GGG G CAG Q CCA	ATG M 1476 GCT A 1530 GTG	CTG L GCC A GAG	TAT Y GTG	CGA R 1485 GGC  G 1539 ATG	GGG G CTG L CTG	AGT S	GTT V 1494 GAC D 1548 GGG	CAA Q GGC	CCC P CTG L GGA	GAC D 1503 GCC A 1557 CCC	TCG S CAA Q Q	GCT A GCC A	L 1512 ATC  I 1566 AAG
I CCC	GTG V TTT F	GTT V 1467 AAC N 1521 CAC H 1575	GCA A TTC F TAT	GGG G CAG Q CCA	ATG M 1476 GCT A 1530 GTG	CTG L GCC A GAG	TAT Y GTG	CGA R 1485 GGC  G 1539 ATG	GGG G CTG L CTG	AGT S	GTT V 1494 GAC D 1548 GGG	CAA Q GGC	CCC P CTG L GGA	GAC D 1503 GCC A 1557 CCC	TCG S CAA Q Q	GCT A GCC A	L 1512 ATC  I 1566 AAG
I CCC	GTG V TTT F	GTT V 1467 AAC N 1521 CAC H 1575	GCA A TTC F TATT Y	GGG G G CAG Q CCA P P 3'	ATG M 1476 GCT A 1530 GTG	CTG L GCC A GAG	TAT Y GTG	CGA R 1485 GGC  G 1539 ATG	GGG G CTG L CTG	AGT S	GTT V 1494 GAC D 1548 GGG	CAA Q GGC	CCC P CTG L GGA	GAC D 1503 GCC A 1557 CCC	TCG S CAA Q Q	GCT A GCC A	L 1512 ATC  I 1566 AAG

Fig. 17(D) (Sheet 3 of 3)

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#### **pAS106**

```
LOCUS
            PAS106.DNA
                         1596 bp
                                    mRNA
                                                    PRI
                                                               06-MAR-1995
            Humanised HMFG1 Fab'2 fused to human DNase I with SV40
DEFINITION
NLS (pAS106)
ACCESSION
NID
KEYWORDS
            DNase I.
SOURCE
            DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
  AUTHORS
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  TITLE
            Recombinant human DNase I reduces the viscosity of cystic.
fibrosis
            sputum
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  JOURNAL
  MEDLINE
            91067672
BASE COUNT
                355 a
                         475 c
                                  452 g
                                           314 t
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACA<u>TGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGGAGCGGC</u>
      781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
      841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
      901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
      961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
     1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
    1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
     1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
     1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
     1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
     1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC
     1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
    1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
    1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
     1561 AAGGGGGGCG GACCCAAAAA GAAGCGCAAG GTTTGA
//
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```
SYN
                                                              25-AUG-2000
            FDDNASE106 1596 BP SS-DNA
LOCUS
DEFINITION
ACCESSION
KEYWORDS
SOURCE
                    Location/Qualifiers
FEATURES
                     join(1..>720,<799..1596)
                     /note="1 to 1596 of PAS106.dna [Split]"
                     721..798
     frag
                     /note="1 to 78 of 102/106linker"
                                                      0 OTHER
                      474 C
                                 452 G 315 T
                355 A
BASE COUNT
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGGAGCGGC
      781 GGGCTGAAGA TCGCAGCCTT CAACATCCAG ACATTTGGGG AGACCAAGAT GTCCAATGCC
      841 ACCCTCGTCA GCTACATTGT GCAGATCCTG AGCCGCTACG ACATCGCCCT GGTCCAGGAG
      901 GTCAGAGACA GCCACCTGAC TGCCGTGGGG AAGCTGCTGG ACAACCTCAA TCAGGACGCA
      961 CCAGACACCT ATCACTACGT GGTCAGTGAG CCACTGGGAC GGAACAGCTA TAAGGAGCGC
     1021 TACCTGTTCG TGTACAGGCC TGACCAGGTG TCTGCGGTGG ACAGCTACTA CTACGATGAT
     1081 GGCTGCGAGC CCTGCGGGAA CGACACCTTC AACCGAGAGC CAGCCATTGT CAGGTTCTTC
     1141 TCCCGGTTCA CAGAGGTCAG GGAGTTTGCC ATTGTTCCCC TGCATGCGGC CCCGGGGGAC
     1201 GCAGTAGCCG AGATCGACGC TCTCTATGAC GTCTACCTGG ATGTCCAAGA GAAATGGGGC
     1261 TTGGAGGACG TCATGTTGAT GGGCGACTTC AATGCGGGCT GCAGCTATGT GAGACCCTCC
     1321 CAGTGGTCAT CCATCCGCCT GTGGACAAGC CCCACCTTCC AGTGGCTGAT CCCCGACAGC
     1381 GCTGACACCA CAGCTACACC CACGCACTGT GCCTATGACA GGATCGTGGT TGCAGGGATG
     1441 CTGCTCCGAG GGGCCGTTGT TCCCGACTCG GCTCTTCCCT TTAACTTCCA GGCTGCCTAT
     1501 GGCCTGAGTG ACCAACTGGC CCAAGCCATC AGTGACCACT ATCCAGTGGA GGTGATGCTG
     1561 AAGGGGGGCG GACCCAAAAA GAAGCGCAAG GTTTGA
11
```

Fig. 18(B)

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```
FDDNASE106 1605 BP SS-DNA
                                                    SYN
LOCUS
                                                              29-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
FEATURES
                    Location/Qualifiers
    frag
                    10..1605
                    /note="1 to 1596 of FdDNase106correct"
                    join(10..>729,<808..1605)
    frag
                     /note="1 to 1596 of PAS106.dna [Split]"
                    730..807
    frag
                     /note="1 to 78 of 102/106linker"
                                          315 T
BASE COUNT
                356 A
                        480 C
                                454 G
                                                     0 OTHER
ORIGIN
       1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
     181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
      301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
     361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
      481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
      541 TGGAACTCAG GCGCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
     661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
     721 AAATCTTGTG ACAAAACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG
     781 GGGAGCGGCG GGCTGAAGAT CGCAGCCTTC AACATCCAGA CATTTGGGGA GACCAAGATG
     841 TCCAATGCCA CCCTCGTCAG CTACATTGTG CAGATCCTGA GCCGCTACGA CATCGCCCTG
     901 GTCCAGGAGG TCAGAGACAG CCACCTGACT GCCGTGGGGA AGCTGCTGGA CAACCTCAAT
     961 CAGGACGCAC CAGACACCTA TCACTACGTG GTCAGTGAGC CACTGGGACG GAACAGCTAT
    1021 AAGGAGCGCT ACCTGTTCGT GTACAGGCCT GACCAGGTGT CTGCGGTGGA CAGCTACTAC
    1081 TACGATGATG GCTGCGAGCC CTGCGGGAAC GACACCTTCA ACCGAGAGCC AGCCATTGTC
    1141 AGGTTCTTCT CCCGGTTCAC AGAGGTCAGG GAGTTTGCCA TTGTTCCCCT GCATGCGGCC
    1201 CCGGGGGACG CAGTAGCCGA GATCGACGCT CTCTATGACG TCTACCTGGA TGTCCAAGAG
    1261 AAATGGGGCT TGGAGGACGT CATGTTGATG GGCGACTTCA ATGCGGGCTG CAGCTATGTG
    1321 AGACCCTCCC AGTGGTCATC CATCCGCCTG TGGACAAGCC CCACCTTCCA GTGGCTGATC
    1381 CCCGACAGCG CTGACACCAC AGCTACACCC ACGCACTGTG CCTATGACAG GATCGTGGTT
    1441 GCAGGGATGC TGCTCCGAGG GGCCGTTGTT CCCGACTCGG CTCTTCCCTT TAACTTCCAG
    1501 GCTGCCTATG GCCTGAGTGA CCAACTGGCC CAAGCCATCA GTGACCACTA TCCAGTGGAG
    1561 GTGATGCTGA AGGGGGGCGG ACCCAAAAAG AAGCGCAAGG TTTGA
11
```

Fig. 18(C)

9 18 27 36 45 54																	
		9			18			27			36			45			54
እጥር	CCA		204	ጥርጥ	ATC	ATC	CTC	TTC		GTA							
AIG	GGA	100															
					I	т.	т		τ.	v	Α	т	A	т	G	v ·	н
M	G	₩	S	С	1	1	ь	r	U	v		•	^	•	•	•	••
																	100
		63			72			81			90		_	99			108
TCC	CAG	GTG	CAG	CTG	GTG	CAG				GAG				CCT	GGG	GCC	TCA
s	0	v	Q.	L	v	Q	S	G	· A	E	v	K	K	P	G	A	S
	-		_,														
		117			126		•	135			144			153			162
CTC.	220		TVCC	WCC.	סעע	COM	ተረጉጥ	CCC	ጥልሮ	ACC	יאדיני			TAC	TGG	ATA	GAG
GIG	AAG	GIG	100	160	AAG	GCI	101	GGC	IFIC								
														v	W	Ψ.	E
v	K	v	S	C.	K	A	S	G	Y	·T	r	5	A	I	**	1	E
											•						
		171			180			189							•		
TGG	GTG	CGC	CAG	GCT	CCA	GGA	AAG	GGC	CTC	GAG	TGG	GTC	GGA	GAG	ATT	TTA	CCT
				·					<del></del>								
w	v	R	0	A	P	G	K	G	L	E	W	v	G	E	I	L	₽
••	•		_			•											
		225			234			243			252			261			270
				man						TTC						CTC	
GGA	AGT	AAT	AAT	TCT	AGA	TAC	AAT	GAG	MMG	110	AAG		CGA				
																17	- m
G	. S	N	N	S	R	Y	N	E	. К	F	K	G	R	V	1	V	r
		279			288			297									
AGA	GAC	ACA	TCC	ACA	AAC	ACA	GCC	TAC	ATG	GAG	CTC	AGC	AGC	CTG	AGG	TCT	GAG
R	· D	T	S	T	N	T	A	Y	M	E	L	s	S	L	R	S	E
		333			342			351			360			369			378
CAC	מיא			TAT	TAC	тст	GCA	AGA	TCC	TAC	GAC	TTT	GCC	TGG	TTT	GCT	TAC
Gric	1.001																
			17	v	v		λ.			Y			A	W	F	A	Y
ם	T	A	v	7		_	A		3	-		•	••	••	•		•
								405			414			423			432
		387															
TGG	GGC	CAA	GGG	ACT	CTG	GTC	ACA	GTC	TCC	TCA	GCC	TCC	ACC	AAG	بافاق	CCA	TUG
W	G	.Q	, G,	T	L:	v	T	,V	S	. "S,	,A.	S.	T.	K	Ģ	P	S
		441			450			459			468			477			486
GTC	TTC	ccc	CTC	GCA	ccc	TCC	TCC	AAG	AGC	ACC	TCT	GGG	GGC	ACA	GCG	GCC	CTG
v	F	Þ	ī.	A	P												L
v	-	•	-	**	•	_	Ū		_								
		400			E 0.4			513	•		522			531			540
		495			504								Omc.			220	
GGC	: TGC	: CTC	GTC	. AAG	GAC	TAC	. TTC	. ccc	GAA	CCG	GIG	ALG	GIG	100	166	ハハ	ICM
G	С	L	ν	K	D	Y	F	P	£	P	v	T	v	S	W	N	S
		549			558												594
GGC	GCC	CTO	ACC	: AGC	GGC	GTO	CAC	: ACC	TTC	CCG	GCT	GTC	CTA	CAG	TCC	TCA	GGA
										P							
J		_	-	_	_	-	-							-			

Fig. 18(C) (Sheet 1 of 3)

		603			612			621			630			639			648
CTC	TAC	TCC	CTC	AGC	AGC	GTG	GTG	ACC	GTG	ccc	TCC	AGC	AGC	TTG	GGC	ĄCC	
				<del></del>							. <b></b>						
L	Y	S.	L	S	S	v	V	T	V	P	s	S	S	L	G	Т	Q
		657			666			675			684			693			702
ACC	TAC		TGC	AAC		TAA	CAC			AGC				GTG		AAG	
T	Y	Ι	С	N <sub>.</sub>	v	N	H	ĸ	P	S	N	T	K	v	D	ĸ	Ř
		711			720			729			738			747			756
GTT	GAG		AAA	TCT		GAC	AAA		CAC	ACA		TGT	GTG	GAG		CCA	
v.	E	P	K	S	С	D	, K	T	H	T	С	С	V	Ē.	. С	P	Ð
		765			774			783			792			801			810
TGC	CCA		CCT	GAA			GGC		CTG	AAG			GCC	TTC		ATC	
С	P	A	P	E	G	S	G	G	L	K	I	A	A	F	N	I	Q
		819			828			837			846			855			864
ACA	TTT		GAG	ACC		ATG	TCC		GCC	ACC		GTC	AGC	TAC		GTG	
T	F	G	E	T	K	M	S	N	A	T	L	V	S	Y	I	A	Q
		873			882			891			900			909			918
ATC	CTG	AGC	CGC	TAC	GAC	ATC	GCC		GTC	CAG		GTC	AGA	GAC	AGC	CAC	
Ι	L	S	R	Y	D	Ι	A	L	V	. Q	E	V	R	D	s	Н	L
		927		•	936			945			954			963		•	972
ACT.	GCC	GIG	GGG	AAG	CTG	CTG	GAC	AAC	CTC	AAT	CAG	GAC	GCA	CCA	GAC	ACC	TAT
T	A	٧	G	K	L	L	D	N	L	N	Q	D	A	P	D	T	Y
		981			990			999		1	1008		;	1017		1	1026
CAC	TAÇ	GTG	GTC	agt	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	AAG	GAG	CGC	TAC	CTG
	~~~"	`															
H	Y	. V	V	s	Ē	₽	L	G	R	N	S	Y	K	E	R	Y	L
	J	1035		:	1044		:	1053		1	1062		:	1071		1	080
TTC	GTG	TAC	AGG	CCT	GAC	CAG	GTC	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT
	v	ν	 R	 Þ		0		٠						 Y	 v.		
-	•	•	•`	•		×	٠	-	••	٠,			•	•	•	Ü	U
	]	1089		:	1098		:	107		3	116		:	1125		3	.134
GGC	TGC	GAG	CCC	TGC	GGG	AAC	GAC	ACC	TTC	AAC	CGA	GAG	CCA	GCC	ATT ·	GTC	AGG
G	C	E	 P	C		N N	D	Т	F		 R	E	P	A		v	Б.
																	•••
		1143			1152			161			170			179			188
TTC	TTC	TCC	CGG	TTC	ACA	GAG	GTC	AGG	GAG	TTT	GCC	ATT	GTT	ccc	CTG	CAT	GCG
·F	F	s	R	F	T	£	v	R	E	F	A	I	·v	P	L	н	A
	_	107					_	<b></b>			<b></b>						
			77.		1206 <b>7</b>			215					. ]	.233		1	242
		1	Fiz	5.	10	)((	<i>L)</i>			- 2	!-						
			Sh														
		- (	JI	CC	r 4	· U	/ ~	"									

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GCC CCG GGG GAC GCA GTA GCC GAG ATC GAC GCT CTC TAT GAC GTC TAC CTG GAT --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---A P G D A V A E I D A L Y D V Y L D 1287 1260 1269 1278 GTC CAA GAG AAA TGG GGC TTG GAG GAC GTC ATG TTG ATG GGC GAC TTC AAT GCG V Q E K W G L E D V M L M G D F N A 1305 1323 1332 1314 1341 GGC TGC AGC TAT GTG AGA CCC TCC CAG TGG TCA TCC ATC CGC CTG TGG ACA AGC G C S Y V R P S Q W S S I R L W T S 1368 1377 1386 1395 CCC ACC TTC CAG TGG CTG ATC CCC GAC AGC GCT GAC ACC ACA GCT ACA CCC ACG PTFQWLIPDSADTTATPT 1413 1422 1431 1440 1449 CAC TGT GCC TAT GAC AGG ATC GTG GTT GCA GGG ATG CTG CTC CGA GGG GCC GTT 1476 1485 1494 1503 GTT CCC GAC TCG GCT CTT CCC TTT AAC TTC CAG GCT GCC TAT GGC CTG AGT GAC V P D S A L P F N F Q A A Y G L S D 1548 1530 1539 CAA CTG GCC CAA GCC ATC AGT GAC CAC TAT CCA GTG GAG GTG ATG CTG AAG GGG Q L A Q A I S D H Y P V E V M L K G 1584 1593 GGC GGA CCC AAA AAG AAG CGC AAG GTT TGA 3" G G P K K K R K V \*

Fig. 18(C) (Sheet 3 of 3)

#### **pAS107**

```
LOCUS
            PAS107.DNA
                         1590 bp
                                    mRNA
                                                               06-MAR-1995
                                                     PRI
            Humanised HMFG1 Fab'2 fused to human DNase I with SV40
DEFINITION
NLS (pAS107)
ACCESSION
NID
KEYWORDS
            DNase I.
SOURCE
            DNase I sequence is from assembled oligos (thus modified c/f
MHDNASE1.dna)
  ORGANISM Homo sapiens
            Eukaryotae; mitochondrial eukaryotes; Metazoa; Chordata;
            Vertebrata; Eutheria; Primates; Catarrhini; Hominidae; Homo.
            Shak, S., Capon, D.J., Hellmiss, R., Marsters, S.A. and Baker, C.L.
  AUTHORS
  TITLE
            Recombinant human DNase I reduces the viscosity of cystic
fibrosis
            sputum
  JOURNAL
            Proc. Natl. Acad. Sci. U.S.A. 87 (23), 9188-9192 (1990)
  MEDLINE
            91067672
BASE COUNT
                354 a
                         474 c
                                  448 q
                                           314 t
ORIGIN
        1 ATGGGATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACA<u>TGCTG TGTGGAGTGC CCACCGTGCC CAGCACCTGA AGGCGGG</u>CTG
```

781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG

1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
1441 CGAGGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG

1441 CGAGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG

1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA

11

\_NLS

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SYN
                                                              25-AUG-2000
                       1590 BP SS-DNA
LOCUS
            FDDNASE107
DEFINITION
ACCESSION
KEYWORDS
SOURCE
FEATURES
                    Location/Qualifiers
                     join(1..>720,<793..1590)
     frag
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                     721..792
     frag
                     /note="1 to 72 of 103/107linker"
                     join (721..>771,<772..792)
     fraq
                     /note="1 to 78 of 102linker [Split]"
                                 448 G
                                                      0 OTHER
                                           315 T
BASE COUNT
                354 A
                         473 C
ORIGIN
        1 ATGGCATGGA GCTGTATCAT CCTCTTCTTG GTAGCAACAG CTACAGGTGT CCACTCCCAG
       61 GTGCAGCTGG TGCAGTCTGG GGCAGAGGTG AAAAAGCCTG GGGCCTCAGT GAAGGTGTCC
      121 TGCAAGGCTT CTGGCTACAC CTTCAGTGCC TACTGGATAG AGTGGGTGCG CCAGGCTCCA
      181 GGAAAGGGCC TCGAGTGGGT CGGAGAGATT TTACCTGGAA GTAATAATTC TAGATACAAT
      241 GAGAAGTTCA AGGGCCGAGT GACAGTCACT AGAGACACAT CCACAAACAC AGCCTACATG
      301 GAGCTCAGCA GCCTGAGGTC TGAGGACACA GCCGTCTATT ACTGTGCAAG ATCCTACGAC
      361 TTTGCCTGGT TTGCTTACTG GGGCCAAGGG ACTCTGGTCA CAGTCTCCTC AGCCTCCACC
      421 AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG
      481 GCCCTGGGCT GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA
      541 GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC AGGACTCTAC
      601 TCCCTCAGCA GCGTGGTGAC CGTGCCCTCC AGCAGCTTGG GCACCCAGAC CTACATCTGC
      661 AACGTGAATC ACAAGCCCAG CAACACCAAG GTGGACAAGA AAGTTGAGCC CAAATCTTGT
      721 GACAAAACTC ACACATGCTG TGTCGAGTGT CCACCGTGTC CAGCACCAGA GGGCGGGCTG
      781 AAGATCGCAG CCTTCAACAT CCAGACATTT GGGGAGACCA AGATGTCCAA TGCCACCCTC
      841 GTCAGCTACA TTGTGCAGAT CCTGAGCCGC TACGACATCG CCCTGGTCCA GGAGGTCAGA
      901 GACAGCCACC TGACTGCCGT GGGGAAGCTG CTGGACAACC TCAATCAGGA CGCACCAGAC
      961 ACCTATCACT ACGTGGTCAG TGAGCCACTG GGACGGAACA GCTATAAGGA GCGCTACCTG
     1021 TTCGTGTACA GGCCTGACCA GGTGTCTGCG GTGGACAGCT ACTACTACGA TGATGGCTGC
     1081 GAGCCCTGCG GGAACGACAC CTTCAACCGA GAGCCAGCCA TTGTCAGGTT CTTCTCCCGG
     1141 TTCACAGAGG TCAGGGAGTT TGCCATTGTT CCCCTGCATG CGGCCCCGGG GGACGCAGTA
     1201 GCCGAGATCG ACGCTCTCTA TGACGTCTAC CTGGATGTCC AAGAGAAATG GGGCTTGGAG
     1261 GACGTCATGT TGATGGGCGA CTTCAATGCG GGCTGCAGCT ATGTGAGACC CTCCCAGTGG
     1321 TCATCCATCC GCCTGTGGAC AAGCCCCACC TTCCAGTGGC TGATCCCCGA CAGCGCTGAC
     1381 ACCACAGCTA CACCCACGCA CTGTGCCTAT GACAGGATCG TGGTTGCAGG GATGCTGCTC
     1441 CGAGGGCCG TTGTTCCCGA CTCGGCTCTT CCCTTTAACT TCCAGGCTGC CTATGGCCTG
     1501 AGTGACCAAC TGGCCCAAGC CATCAGTGAC CACTATCCAG TGGAGGTGAT GCTGAAGGGG
     1561 GGCGGACCCA AAAAGAAGCG CAAGGTTTGA
11
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Fig. 19(B)

WO 01/74905 PCT/GB01/01324

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FDDNASE107
                                                    SYN
LOCUS
                         1599 BP SS-DNA
                                                              29-AUG-2000
DEFINITION
ACCESSION
KEYWORDS
SOURCE
FEATURES
                     Location/Qualifiers
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     frag
                     /note="1 to 1590 of FdDNase107correct"
                     join(10..>729,<802..1599)
     frag
                     /note="1 to 1590 of PAS107.dna [Split]"
                     730..801
     frag
                     /note="1 to 72 of 103/107linker"
                     join(730..>780,<781..801)
     frag
                     /note="1 to 78 of 102linker [Split]"
BASE COUNT
                         479 C
                                 450 G
                                           315 T
ORIGIN
       1 GCCGCCACCA TGGGATGGAG CTGTATCATC CTCTTCTTGG TAGCAACAGC TACAGGTGTC
       61 CACTCCCAGG TGCAGCTGGT GCAGTCTGGG GCAGAGGTGA AAAAGCCTGG GGCCTCAGTG
      121 AAGGTGTCCT GCAAGGCTTC TGGCTACACC TTCAGTGCCT ACTGGATAGA GTGGGTGCGC
      181 CAGGCTCCAG GAAAGGGCCT CGAGTGGGTC GGAGAGATTT TACCTGGAAG TAATAATTCT
      241 AGATACAATG AGAAGTTCAA GGGCCGAGTG ACAGTCACTA GAGACACATC CACAAACACA
      301 GCCTACATGG AGCTCAGCAG CCTGAGGTCT GAGGACACAG CCGTCTATTA CTGTGCAAGA
      361 TCCTACGACT TTGCCTGGTT TGCTTACTGG GGCCAAGGGA CTCTGGTCAC AGTCTCCTCA
      421 GCCTCCACCA AGGGCCCATC GGTCTTCCCC CTGGCACCCT CCTCCAAGAG CACCTCTGGG
      481 GGCACAGCGG CCCTGGGCTG CCTGGTCAAG GACTACTTCC CCGAACCGGT GACGGTGTCG
      541 TGGAACTCAG GCGCCCTGAC CAGCGGCGTG CACACCTTCC CGGCTGTCCT ACAGTCCTCA
      601 GGACTCTACT CCCTCAGCAG CGTGGTGACC GTGCCCTCCA GCAGCTTGGG CACCCAGACC
      661 TACATCTGCA ACGTGAATCA CAAGCCCAGC AACACCAAGG TGGACAAGAA AGTTGAGCCC
      721 AAATCTTGTG ACAAAACTCA CACATGCTGT GTCGAGTGTC CACCGTGTCC AGCACCAGAG
      781 GGCGGGCTGA AGATCGCAGC CTTCAACATC CAGACATTTG GGGAGACCAA GATGTCCAAT
      841 GCCACCTCG TCAGCTACAT TGTGCAGATC CTGAGCCGCT ACGACATCGC CCTGGTCCAG
      901 GAGGTCAGAG ACAGCCACCT GACTGCCGTG GGGAAGCTGC TGGACAACCT CAATCAGGAC
      961 GCACCAGACA CCTATCACTA CGTGGTCAGT GAGCCACTGG GACGGAACAG CTATAAGGAG
     1021 CGCTACCTGT TCGTGTACAG GCCTGACCAG GTGTCTGCGG TGGACAGCTA CTACTACGAT
     1081 GATGGCTGCG AGCCCTGCGG GAACGACAC TTCAACCGAG AGCCAGCCAT TGTCAGGTTC
     1141 TTCTCCCGGT TCACAGAGGT CAGGGAGTTT GCCATTGTTC CCCTGCATGC GGCCCCGGGG
     1201 GACGCAGTAG CCGAGATCGA CGCTCTCTAT GACGTCTACC TGGATGTCCA AGAGAAATGG
     1261 GGCTTGGAGG ACGTCATGTT GATGGGCGAC TTCAATGCGG GCTGCAGCTA TGTGAGACCC
     1321 TCCCAGTGGT CATCCATCCG CCTGTGGACA AGCCCCACCT TCCAGTGGCT GATCCCCGAC
     1381 AGCGCTGACA CCACAGCTAC ACCCACGCAC TGTGCCTATG ACAGGATCGT GGTTGCAGGG
    1441 ATGCTGCTCC GAGGGGCCGT TGTTCCCGAC TCGGCTCTTC CCTTTAACTT CCAGGCTGCC
    1501 TATGGCCTGA GTGACCAACT GGCCCAAGCC ATCAGTGACC ACTATCCAGT GGAGGTGATG
     1561 CTGAAGGGG GCGGACCCAA AAAGAAGCGC AAGGTTTGA
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Fig. 19(C)

							-	<i>y</i> <b>U</b> ,	<i>,</i>	L								
			9			18			27			36			45			54
5,	ATG	GGA		AGC	TGT		ATC	CTC	TTC	TTG	GTA	GCA	ACA	GCT	ACA	GGT	GTC	CAC
	M	G	Ŵ	s	С	I	I	L	F	L	V	Α	Т	A	Ť	G	V	н
	•		۲,			72			81			90			99			108
	ጥርር	CAG	63 GTG	CAG	CTG		CAG	TCT		GCA	GAG			AAG		GGG	GCC	
	s	Q	v	Q	L	v	Q	S	G	A	E	V	K	K	P	G	Α	s
						126			125			144			153			162
	Calc	ממ		TCC											_			
	v	K	v	s	C	ĸ	A	S	G	Y	T	F	S	Α	Y	W	I	E
		•							100			198			207			216
	TCC	CTC	171	CAG	CCT	180	GGA	AAG	189 GGC		GAG						TTA	
	W	v	R	Q	A	P	G	ĸ	G	L	E	W	v	G	E	Ι	L	P
									242			252			261			270
	001	3 OIII	225	TAA '	TV-TT	234		<b>ከ</b> ውጥ	243		•	252 AAG		CGA			GTC	
	GGA	AGT		AAI														
	G	s	N	N	S	R	¥	N	E	K	F	ĸ	G	R	v	T	V	Ŧ
												200			315			224
		C N C	279	TCC	አሮአ	288		ccc	297 יער									324 GAG
	AGA		. ACA															
	R	D	T	s	T	N	T	A	Y	M	E	L	s	S	L	R	s	E
									251			260			369			378
	CNC	י אריא	333	GTC	יי מיי	342 242		י פרא	351 AGA		TAC	360 GAC		GCC				
		. ACA																
	D	T	A	v	Y	Y	С	A	R	S	Y	D	F	A	W	F	A	Y
												44.4			422			422
	mor		387	, GGG	י אריים	396 2000		מרמי			י חרים	414 GCC		ACC	423 AAG		CCA	432 TCG
	W	G	. Q	G	Ţ	L	, <b>V</b>	T	, <b>v</b>	ŗS	S	A	Ş	T	K.	_ <b>G</b> ,	P	S
									450			460		•	477			486
	GTO		441	ן ר	· cci	450		י זיריר	459 244 -		: ACC	468. TCT:		GGC				CTG
	·v	F	P	L	A	P	S	s	K	S	T	s	G	G	T	A	Α	L
									E 2 2			522			531	•	•	540
	CCC	~ n~		5 5 CTPC							cce	522 GTG		GTG			AAC	TCA
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	G	С	L	V	K	D	Y	F	P	E	P	V	T	. V	s	W	N	s
				_						,		576			505			504
		~~	54!			558 200		ב רשר	567 204			576 GCT		CT2	585 CAG		TCA	594 GGA
							<b>-</b> -											
	G	A	L	T	s	G	V	н	T	F	P	A	v	£	Q	s	s	G
			_			41	1	<b>~</b> 1				1 -						

Fig. 19(D) (Sheet 1 of 3)

		C03			612			·· —			620			639			648
CTC	ጥልቦ	603 TCC	CTC				CAC								GGC		
L	Y	s	L	s	s	v	V	т	v	P	S	s ·	s	L	G	T	Q
		657			666			675,						693		<u>.</u>	702
ACC	TAC	ATC	TGC	AAC	GTG	AAT	CAC	AAG	CCC	AGC	AAC	ACC	AAG	GTG.	GAC	AAG	AAA
т	 Y		c	 N	v	N	н	ĸ		s	N	T	K	v	D	к	к
1	1	1	C	14	٧	.14	n	K	-	-		• .	K	. •	-		
		711			720			729			738			747			756
GTT	GAG	CCC.	AAA	TCT	TGT	GAC	AAA	ACT	CAC	ACA	TGC	TGT	GTG	GAG	TGC	CCA	CCG
v	E	P	K	S	С	D	ĸ	T	H	T	С	С	v	E	С	₽.	P
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m~~	CCA	765	OCE.	C	774					CCA	792	and A	אאר	801	CAG	מסמ	810
1GC																	
С	P	A	P.	E	G	Ġ	L	K	I	A	A	F	N	Ī	Q	T	F
											•						
		819			828						846			855			864
GGG	GAG	ACC	AAG	ATG	TCC	TAA	GCC	ACC	CTC	GTC	AGC	TAC	ATT	GTG	CAG	ATC	CTG
					- <del>-</del> -				L	v	 s	 Y		v	Q		L L
G	E	T	K	M	5	N	A	1	b	v	3	1	1	v	Q	1	r
		873			882			891			900			909			918
AGC	CGC	TAC	GAC	ATC	GCC	CTG	GTC	CAG	GAG	GTC	AGA.	GAC	AGC	CAC	CTG	ACT	GCC
					·								<del></del>				
s	R	Y	D	I	A	L	v	Q	E	v	R	D	S	H	L	T	A
		007			02.5			945			954			963			972
CTG	ccc	927	CTC	CALC	936 GAC	244	CTC		CAG	GAC		CCA	CAC		TAT	CAC	
ν	G	ĸ	L	L	D	Ŋ	L	N	Q	D	A	P	D	T	Y	н	Y
		981			990									1017			10.26
GTG	GTC	AGT	GAG	CCA	CTG	GGA	CGG	AAC	AGC	TAT	·AAG	GAG	CGC	TAC	CTG	TTC	GTG
v	v	S	E	P	L	 G	R	N	S	Y	ĸ	E	R	Y	L	F	v
•	•		_	_	_			-	-	_							
		1035		:	1044		:	1053		:	1062			1071		:	1080
TAC	AGG	CCT	GAC	CAG	GTG	TCT	GCG	GTG	GAC	AGC	TAC	TAC	TAC	GAT	GAT	GGC	TGC
															D		
Y	R	P	D	Q	V	S	Α	V	ע	S	Y	Y	Y	ט	ט	G	C
		1089			1098			1107			1116		1	1125			1134
.GAG												•			AGG		
E	P	С	G	N.	D	T	F	N	R	E	P	Α	I	V	R	F	F
		1147			1150			1161			120			1170			1100
ሞርር					1152 GTC										GCG		CCG
s															A		
												•					
		1197			1206			1215		1	1224		1	1233		1	1242
			C:	~ ·	1	$\Omega Z$	D	1									

Fig. 19(D) (Sheet 2 of 3)

GGG	GAC	GCA	GTA	GCC	GAG	ATC	GAC	GCT	CTC	TAT	GAC	GTC	TAC	CTG	GAT	GTC	CAA	
					<u></u>													
G	D	A	V	A	E	I	D	A	L	Y	D	v	Y	L	D	V	Q	
• •							_		•	_				202			200	
		251		1	260		` 1	L269			.278		1	.287			1296	
GAG	AAA	TGG	GGC	TTG	GAG	GAC	GTC	ATG	TTG	ATG	GGC	GAC	TIC	AAT.	GCG	GGC	TGC	
E	K	W	G	L	E	D	V	М	L	М	G	D	F	N	А	G	C	
																	1250	
		1305		1	1314			1323			232	, ama	mac	1341	200	000	1350	•
AGC	TAT	GTG	AGA	CCC	TCC	CAG	TGG	TCA	TCC	ATC	CGC	CTG	TGG	ACA	AGC	CCC	ACC	
S	Y	V	R	P	S	Q	W	S	S	1	ĸ	L	W	÷	3	F	1	
						•					206			1205	٠.		1404	
		1359			1368													
TTC	CAG	TGG	CTG	ATC	ccc	GAC	AGC	GCT	GAC	ALC	ACA	GCT	ACA		ACG			
												A	T.	Þ	Ţ	н	C	
F	Q	W	T.	T	P	ע	5	A	D	-	•		•	•	•	••	Ū	
										-								
		1412			1422			1431		:	1440			1449			1458	
ccc	ጥልጥ	1413 GAC	AGG	ATC	1422 GTG	GTT	GCA	1431 GGG	ATG	CTG	L440 CTC	CGA	GGG	1449 GCC	GTT	GTT	1458 CCC	
GCC	TAT	1413 GAC	AGG	ATC	GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT	GTT	CCC	
	TAT	GAC	AGG	ATC	GTG	GTT	GCA	GGG	ATG	CTG	CTC	CGA	GGG	GCC	GTT 	GTT 		
	TAT  Y	GAC  D	AGG  R	ATC 	GTG  V	V 	GCA  A	GGG  G	ATG  M	CTG  L	CTC L	CGA  R	GGG  G	GCC A	GTT  V	GTT  V		
 A	TAT  Y	GAC  D 1467	AGG  R	ATC  I	GTG  V 1476	V 	GCA A	GGG  G 1485	ATG  M	CTG  L	CTC  L 1494	CGA R	GGG  G	GCC  A 1503	V 	GTT  V	CCC P 1512	
 A	TAT  Y	GAC  D 1467	AGG  R	ATC  I	GTG  V 1476	V 	GCA A	GGG  G 1485	ATG  M	CTG  L	CTC  L 1494	CGA R	GGG  G	GCC  A 1503	V 	GTT  V	CCC P 1512	
A GAC	TAT Y TCG	GAC D 1467 GCT	AGG R CTT	ATC I CCC	GTG  V 1476 TTT	GTT V	GCA A TTC	GGG  G 1485 CAG	ATG M GCT	L GCC	CTC L 1494 TAT	CGA R R	GGG G G CTG	GCC A 1503 AGT	V GAC	V CAA	P 1512 CTG	
A GAC	TAT Y TCG	GAC D 1467 GCT	AGG R CTT	ATC I CCC	GTG  V 1476 TTT	GTT V	GCA A TTC	GGG  G 1485 CAG	ATG M GCT	L GCC	CTC L 1494 TAT	CGA R R	GGG G G CTG	GCC A 1503 AGT	V GAC	V CAA	P 1512 CTG	
A GAC	TAT Y TCG	GAC D 1467 GCT	AGG R CTT	ATC I CCC	GTG  V 1476 TTT	GTT V  AAC	GCA A TTC	GGG  G 1485 CAG  Q	ATG M GCT A	CTG L GCC A	CTC L 1494 TAT Y	CGA R R GGC	GGG G G CTG  L	GCC A 1503 AGT S	GTT V GAC	CAA	P 1512 CTG  L	
A GAC	TAT Y TCG	GAC D 1467 GCT A	AGG R CTT	ATC I CCC	GTG  V 1476 TTT  F	GTT V AAC	GCA A TTC	GGG  G 1485 CAG  Q	ATG M GCT A	CTG L GCC A	CTC L 1494 TAT Y	CGA R R GGC	GGG G CTG	GCC  A 1503 AGT  S	GTT V GAC	CAA	CCC P 1512 CTG  L	
A GAC	TAT Y TCG	GAC D 1467 GCT A	AGG R CTT	ATC I CCC	GTG  V 1476 TTT  F	GTT V AAC	GCA A TTC	GGG G 1485 CAG Q 1539	ATG M GCT A	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC GGC	GGG G CTG	GCC  A 1503 AGT  S	GTT V GAC	CAA CAA GGC	CCC P 1512 CTG  L 1566 GGA	
GAC	TAT Y TCG	GAC  D  1467 GCT  A  1521 GCC	AGG R CTT L	I CCC	GTG V 1476 TTT F 1530 GAC	AAC	GCA A TTC F	GGG G 1485 CAG Q 1539 CCA	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC ATG	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	
GAC	TAT Y TCG	GAC  D  1467 GCT  A  1521 GCC	AGG R CTT L	I CCC	GTG V 1476 TTT F 1530 GAC	AAC	GCA A TTC F	GGG G 1485 CAG Q 1539 CCA	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC GGC	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	
GAC	TAT Y TCG S CAA	GAC D 1467 GCT A 1521 GCC	AGG R CTT L ATC	ATC I CCC P AGT	GTG V 1476 TTT F 1530 GAC	AAC N CAC	GCA A TTC F	GGG G 1485 CAG Q 1539 CCA	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC ATG	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	
GAC	TAT Y TCG S CAA	GAC D 1467 GCT A 1521 GCC A	AGG R CTT L ATC	ATC I CCCC P AGT	GTG V 1476 TTT F 1530 GAC D	AAC N	GCA A TTC F TAT	GGG G 1485 CAG Q 1539 CCA P	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC ATG	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	
GAC	TAT Y TCG S CAA	GAC D 1467 GCT A 1521 GCC	AGG R CTT L ATC	ATC I CCCC P AGT	GTG V 1476 TTT F 1530 GAC D 1584 AAG	AAC N CAC	GCA A TTC F TAT Y	GGG G 1485 CAG Q 1539 CCA P	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC ATG	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	
GAC	TAT Y TCG S CAA	GAC D 1467 GCT A 1521 GCC A	R CTT L ATC	I CCCC P AGT	GTG V 1476 TTT F 1530 GAC D 1584 AAG	AACO N CACO H	GCA A TTC F TATT Y	GGG G 1485 CAG Q 1539 CCA P	ATG M GCT A GTG	CTG L GCC A GAG	CTC L 1494 TAT Y 1548 GTG	GGC GGC ATG	GGG G CTG L CTG	GCC  A 1503 AGT  S 1557 AAG	GAC D GGG	CAA CAA GGC	CCC P 1512 CTG L 1566 GGA	

Fig. 19(D) (Sheet 3 of 3)

## Mammalian expression of humanised HMFG1-D Nase constructs

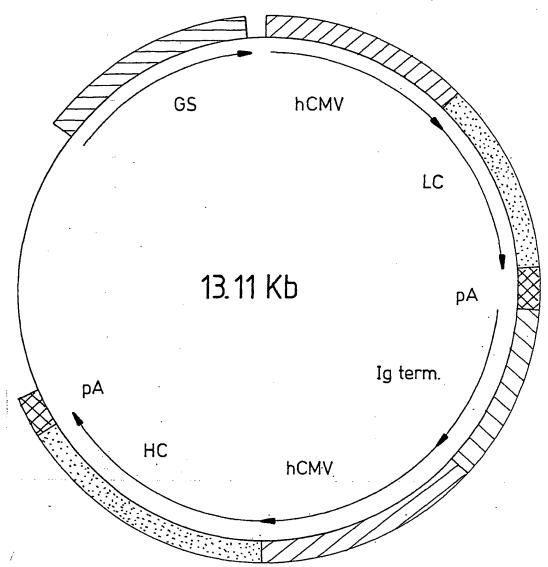


Fig. 20

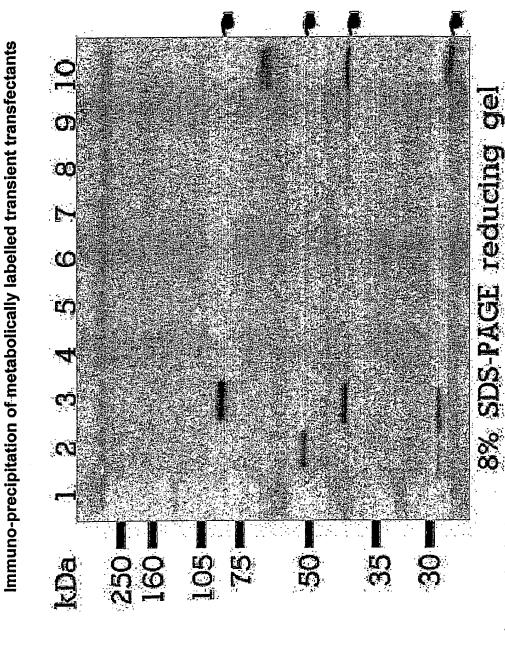
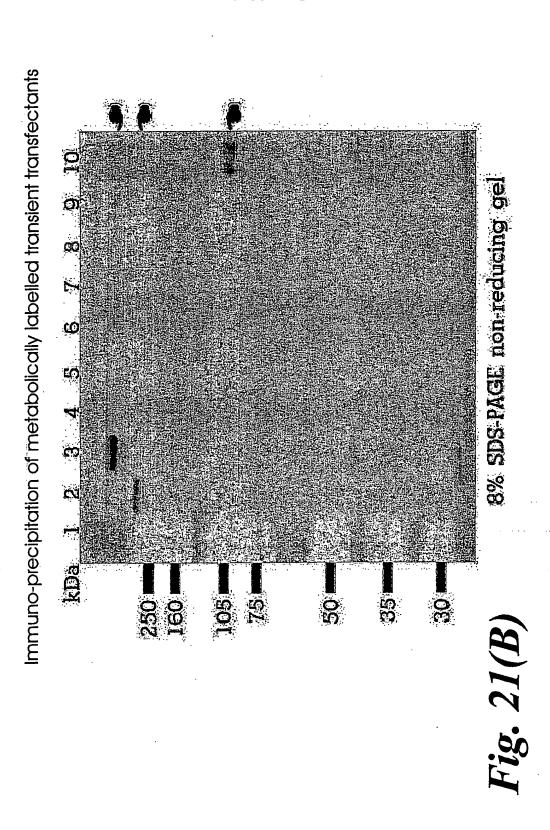
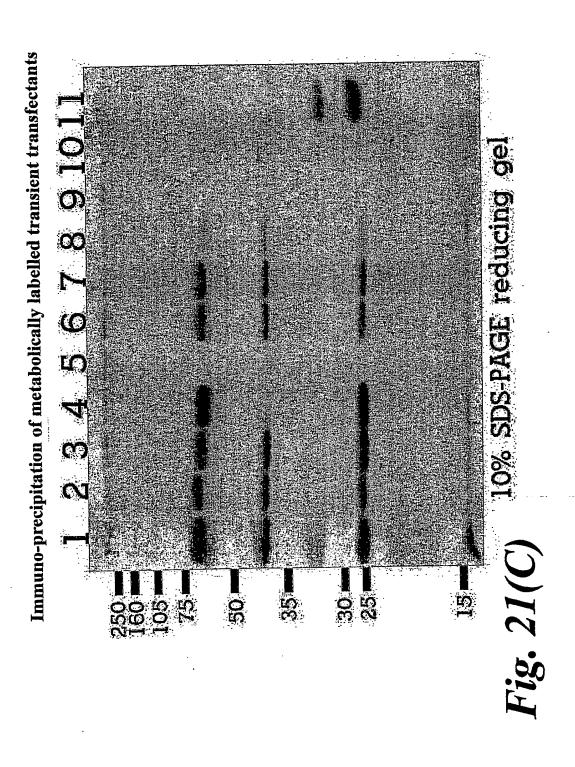


Fig. 21(4)





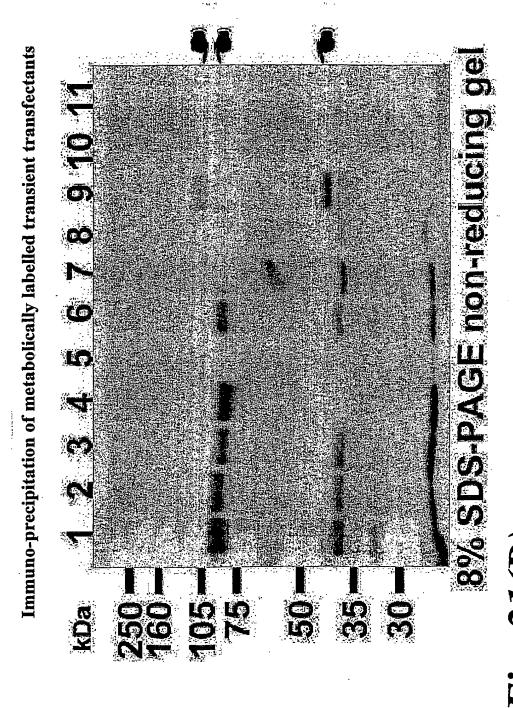
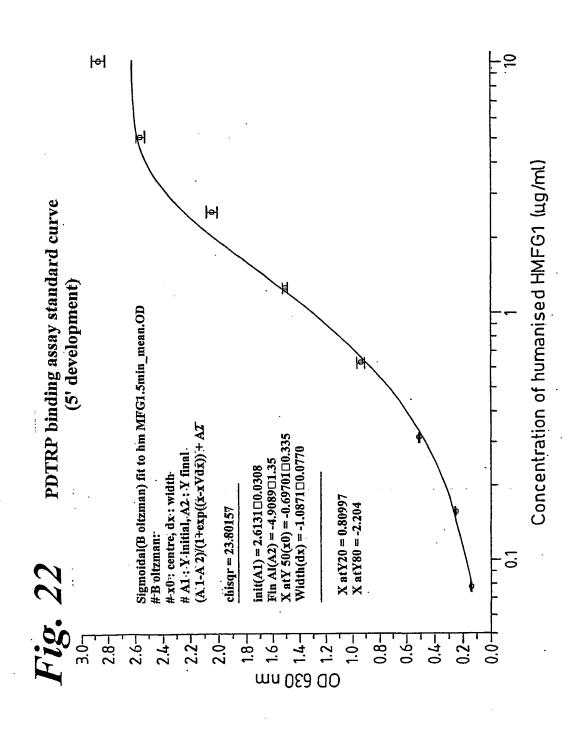


Fig. 21(D)



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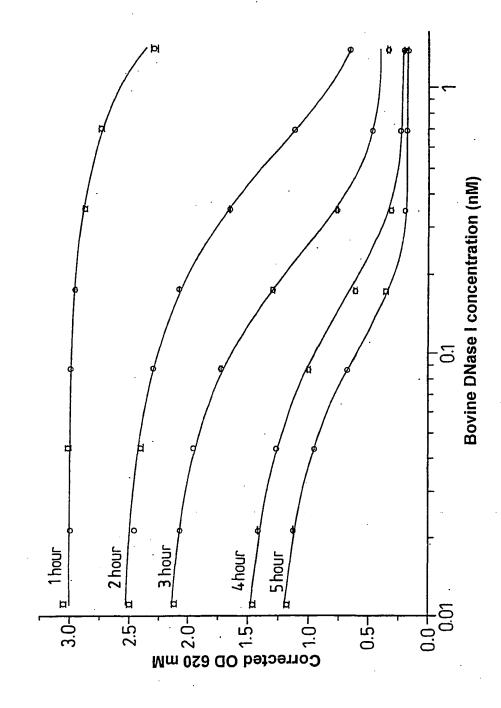
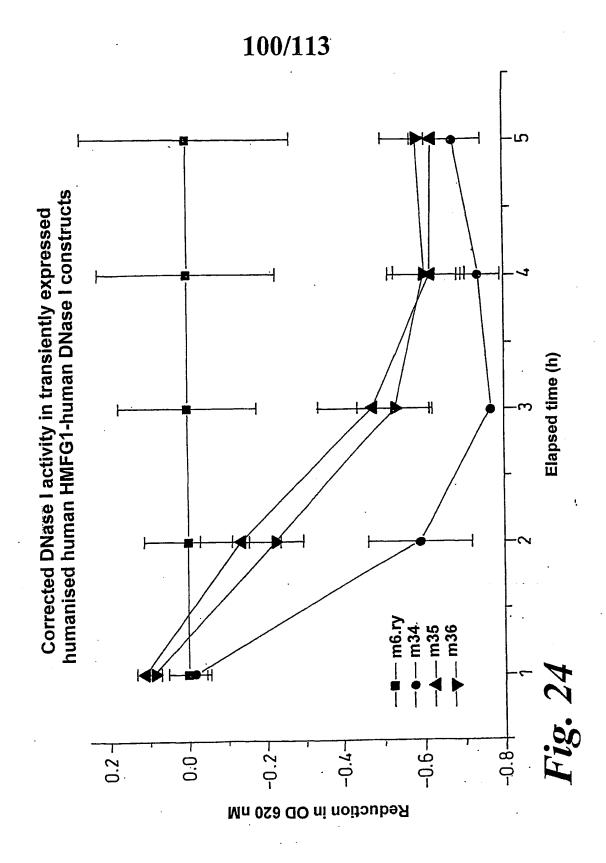
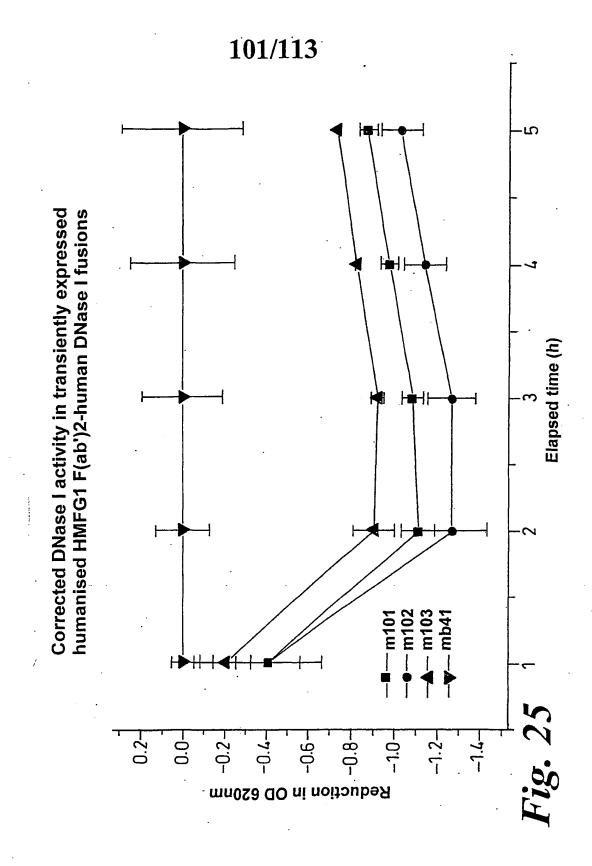


Fig. 23

Corrected bovine DNase I standard curves

at various time points





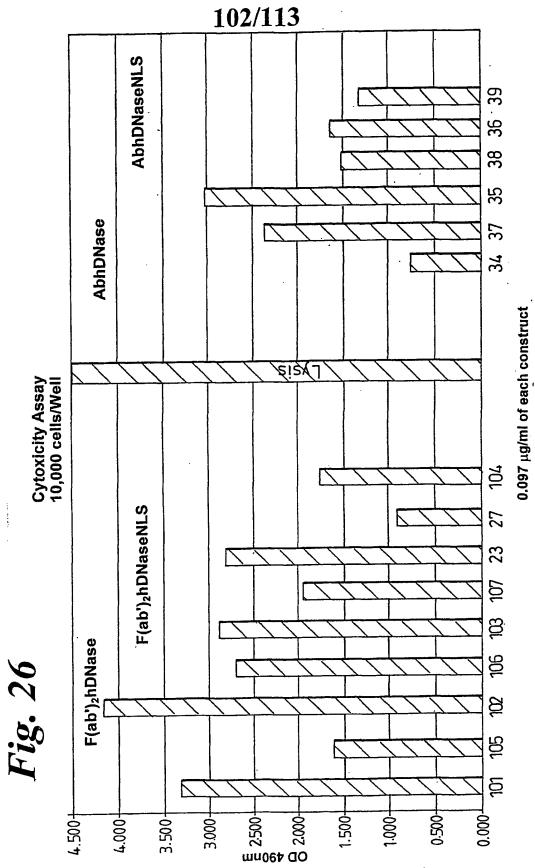
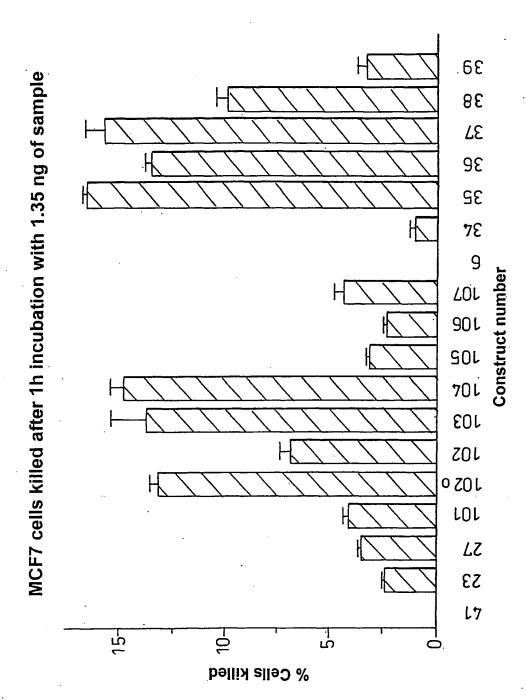
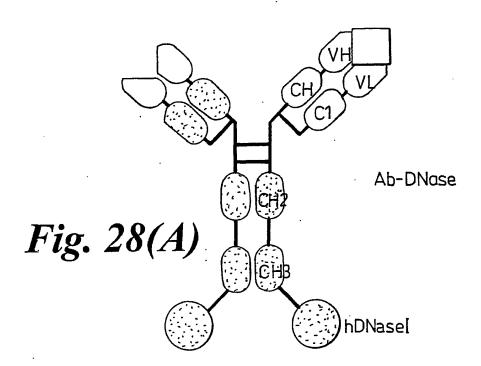


Fig. 27

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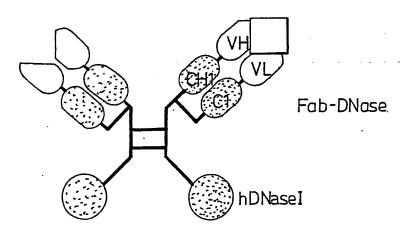
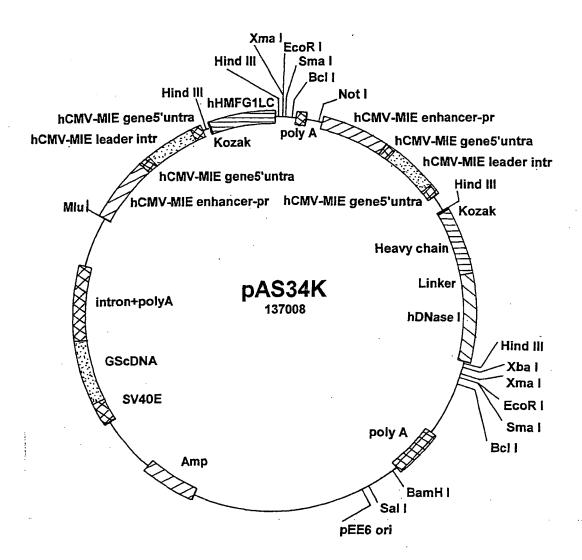
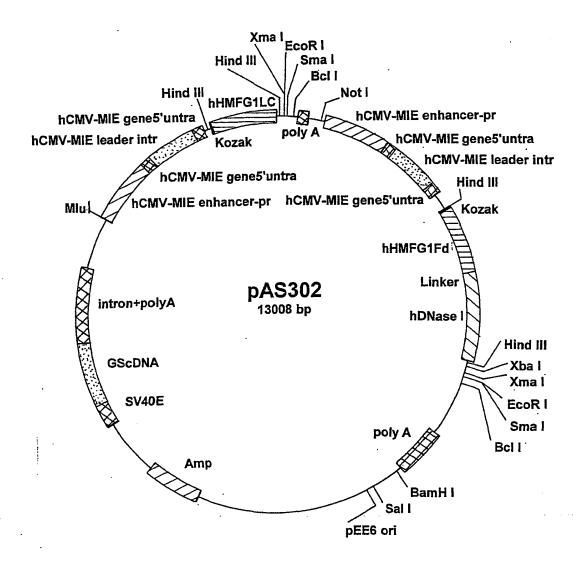


Fig. 28(B)



**Ab-DNase** 

Fig. 29



Fab-DNase

Fig. 30

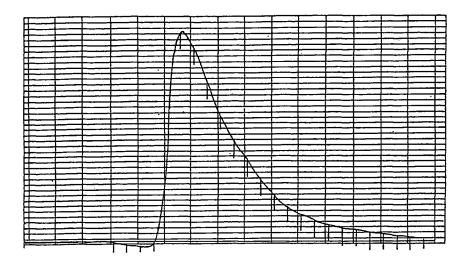


Fig. 31(A)

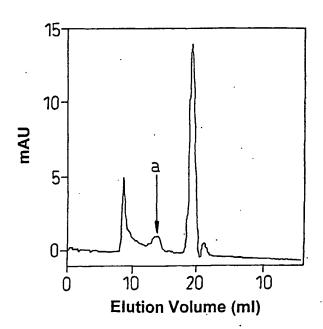
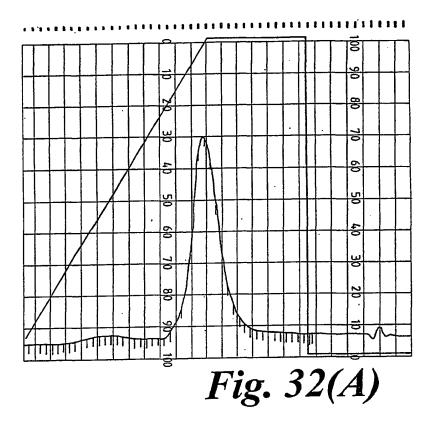
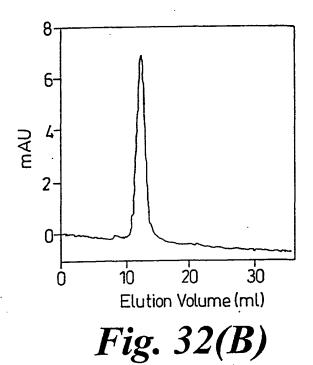
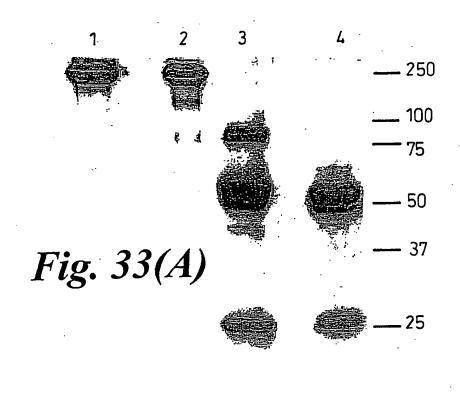


Fig. 31(B)





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 $-75$ 
 $-50$ 

Fig. 33(B)
 $-37$ 

# Bovine DNase I standard curves at various time points

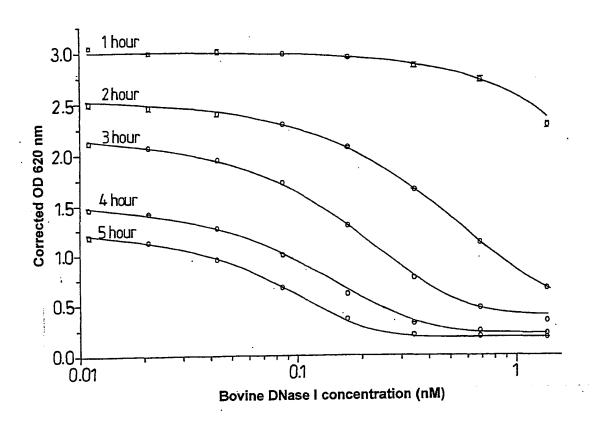


Fig. 34(A)

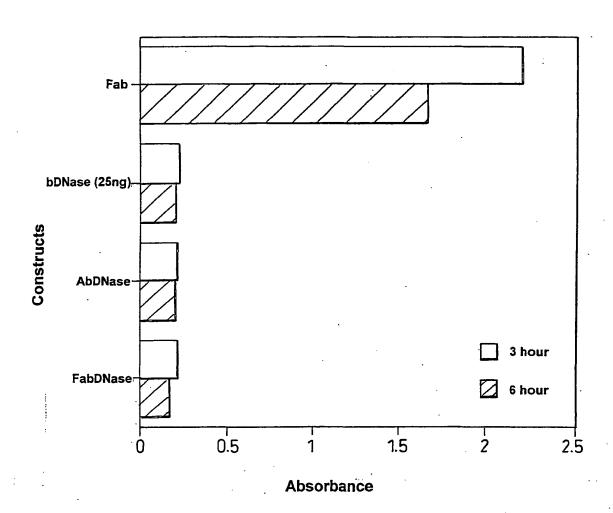


Fig. 34(B)

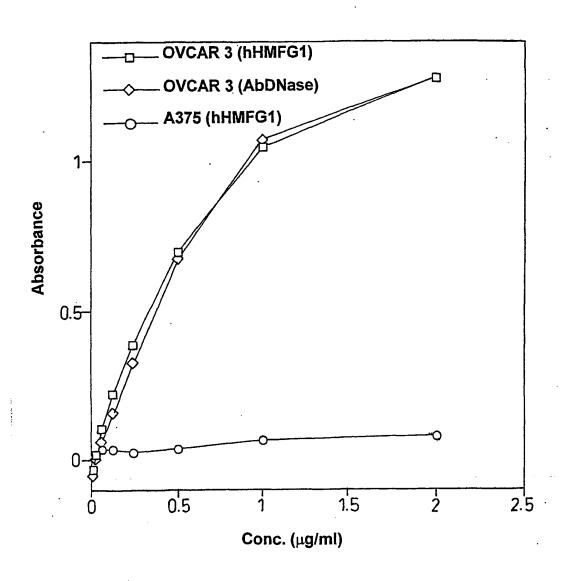


Fig. 35(A)

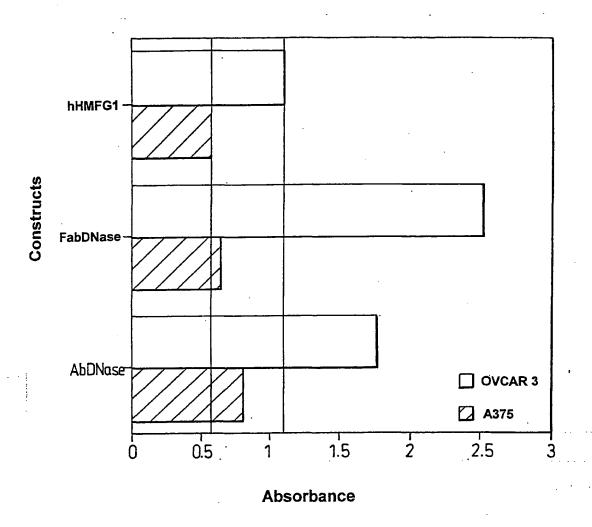


Fig. 35(B)

#### INTERNATIONAL SEARCH REPORT

ational Application No PCT/GB 01/01324

A. CLASSIFICATION OF SUBJECT MATTER IPC 7 C07K16/18 C12M C12N15/63 C07K16/46 C12N9/22 C12N15/62 //co7K19/00 A61K38/43 C12N15/85 A61K39/395 According to International Patent Classification (IPC) or to both national classification and IPC B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) CO7K C12N IPC 7 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the International search (name of data base and, where practical, search terms used) BIOSIS, EPO-Internal, WPI Data, MEDLINE, PAJ C. DOCUMENTS CONSIDERED TO BE RELEVANT Relevant to claim No. Citation of document, with indication, where appropriate, of the relevant passages Category ' 1-12,14, "A DNase I based YOUNG ROBERT J ET AL: X 15,17, immunotoxin for tumor therapy." 21,23, PROCEEDINGS OF THE AMERICAN ASSOCIATION 25, FOR CANCER RESEARCH ANNUAL 28-35, no. 41, March 2000 (2000-03), page 289 37,38 XP001008862 91st Annual Meeting of the American Association for Cancer Research.; San Francisco, California, USA; April 01-05, 2000, March, 2000 ISSN: 0197-016X abstract -/--Patent family members are listed in annex. Further documents are listed in the continuation of box C. X \*T\* later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance invention "E" earlier document but published on or after the International "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled to the O document referring to an oral disclosure, use, exhibition or in the art. document published prior to the international filing date but later than the priority date claimed \*&\* document member of the same patent family Date of mailing of the International search report Date of the actual completion of the international search 16/08/2001 6 August 2001 Authorized officer Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 Montrone, M

page 1 of 2

#### INTERNATIONAL SEARCH REPORT

ational Application No
PCT/GB 01/01324

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	ation) DOCUMENTS CONSIDERED TO BE RELEVANT  Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	LINARDOU H. ET AL.: "Deoxyribonuclease I (DNase I). A novel approach for targeted cancer therapy." CELL BIOPHYS., vol. 24-25, 1994, page 243-248 XP001012902 abstract page 244, paragraphs 2-4 page 245, paragraph 4 page 246; figure 1 page 247, paragraphs 2,3,5	15,16, 18,19
<b>Y</b>	WO 92 04380 A (UNILEVER PLC; UNILEVER NV (NL)) 19 March 1992 (1992-03-19) cited in the application abstract page 4, line 30 - line 27 page 6, line 6-26 page 8, line 30 -page 9, line 7 page 9, line 11-28 page 10, line 1-6 page 10, line 33 -page 11, line 3 page 12, line 13-35 page 14, line 14-16 page 15, line 15-30 page 16, line 15-20 page 17, line 6-14	1-19,21, 23,25, 27-38
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#### FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 20,22,24,26,38

Present claims 20, 24 and 38 relate to an extremely large number of possible compounds. In fact, the claims contain so many options, variables, possible permutations and provisos that a lack of clarity within the meaning of Article 6 PCT arises to such an extent as to render a meaningful search of the claims impossible. Moreover, a search for the subject-matter of claims 22 and 26 has not been carried out since it was not possible to identify the corresponding SEQ.ID.NO. of fig. 14(c). Consequently, the search has been carried out for those parts of the application which do appear to be clear, namely 1 to 19, 21, 23, 25, 27 to 37.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

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